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THE PRODUCTION AND SELECTION OF A FAMILY OF STRAINS IN PENICILLIUM CHRYSOGENUM [1]

M. P. Backus and J. F. Stauffer

(with 4 figures)

A program of research dealing with variation in *Penicillium notatum* Westling and *P. chrysogenum* Thom has now been in progress in the writers' laboratories for over ten years. Begun as one of several projects set up by the War Production Board in its monumental effort to increase production of the then new drug, penicillin, to meet war needs, the program was continued for one year (1944–45) under a grant obtained from the Research Committee of the University of Wisconsin Graduate School, and thereafter with support from various segments of the American antibiotics industry. Initially geared to the sole objective of securing strains of the mold capable of higher yields of the drug, the program was soon modified to emphasize fundamental studies on variability in the fungi in question. Strain development work has at no time been completely discontinued, but at the termination of the government contract it was made subordinate to the broader purposes of an academic research enterprise.

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[Mycologia for May–June (47: 275–428) was issued June 17, 1955]

Beginning during the war period and continuing into the two later phases through which the project has progressed, the writers have worked in close conjunction with other groups conducting penicillin research at the University of Wisconsin. The relationship with the Department of Biochemistry has been particularly close, and the writers are especially indebted to Professors W. H. Peterson, M. J. Johnson, and M. A. Stahmann of that Department for their invaluable cooperation. A succession of graduate students—O. H. Calvert, Eugene Dulaney, Sally Kelly, Bruce Churchill, F. Roegner, T. H. Campbell, Roy Curtis, W. F. Whittingham, and James Grosklags—have assisted in the work; and each of these has played an important part in carrying out certain phases of the investigation. Dr. H. C. Greene of the Botany Department also participated in the project briefly during the time when operations were being carried on under the government contract.

Accounts of the whole general wartime penicillin research program in America, including a consideration of the strains of the mold developed and used during that period, have been published by Coghill and Koch (**12**), Raper (**39, 40**), Raper and Alexander (**41**), Peterson (**36**), Thom (**54**), Florey *et al.* (**15**), Perlman (**35**), and others. The details need not be repeated here. Suffice it to state that although a variety of new strains were secured in this laboratory during the writers' brief participation in the War Production Board program, none was of sufficient merit to be ranked among the best strains in circulation at that time.

In 1945, however, a very superior strain of *P. chrysogenum* was developed at the University of Wisconsin (**4**) and in the further course of operations an outstanding "family" of strains, stemming from that notable variant, has emerged. It is the purpose of the present communication to give an account of this "Wisconsin Family," with special emphasis on key strains and how they were obtained, as background for a discussion of some of the more specialized phases of the study of variability to be reported later. Reports dealing with certain aspects of the general program have already been published (**1, 45, 49, 50**).

THE ANCESTRAL STOCK

The first penicillin produced on an industrial scale was obtained by surface culture methods, and the organism most extensively employed in these pioneer days was a strain of *P. notatum* known as *NRRL 1249·B21* (**41, 42**). This was a derivative of the original culture contributed by Fleming. However, by the time the writers began their work on variability in penicillin-producing molds, industry in America was shifting

to production of the antibiotic in submerged culture. At the U.S.D.A. Northern Regional Research Laboratory, which took the lead in penicillin research in this country when the problem of large-scale production of the drug was first brought to America in 1941, a notable strain of *P. notatum, NRRL 832,* had been brought to light and shown to be capable of giving reasonably good yields of penicillin when grown submerged in aerated containers. Thus strain *NRRL 832* opened the way for development of the tank fermentation method of producing the antibiotic. *NRRL 832* was one of the strains on which the authors began their studies. Since, however, the Wisconsin family of strains to be described in this communication sprang from other stock, strain *NRRL 832* need not be considered further here.

Strains NRRL 1951 and NRRL 1951·B25

In the search for better stocks of penicillin-producing mold, an outstanding step forward was taken in 1943 when Raper and Alexander isolated a strain of *P. chrysogenum* which they designated as *NRRL 1951* (**41**). This strain was selected for further study by its discoverers because in initial tests it gave, in submerged culture, yields somewhat better than those obtained from *NRRL 832.* It is notable, chiefly, however, as the progenitor of a long line of interesting and important descendants, including the entire "Wisconsin Series." Since the middle of 1944 it would appear that descendants of this famous isolate have been the chief source of the world's supply of penicillin.

A large number of spontaneously-occurring variants derived from *NRRL 1951* were selected and studied by Raper and Alexander. One outstanding derivative, *NRRL 1951·B25,* was obtained in two steps. The exact mode of origin of this variant, also its cultural and microscopic characteristics and fermentation behavior are recorded in a series of publications (**17, 30, 31, 41, 42**). In submerged culture, strain *NRRL 1951·B25* gave penicillin yields up to 250 O.U./ml—two to three times the amount obtainable from its wild-type ancestor or from *P. notatum 832*—and quickly began to supplant strain *832* for the production of penicillin when released for industrial use. Although Raper and Alexander endeavored to obtain still higher-yielding strains by isolating variants from *1951·B25,* they were unsuccessful in this attempt (**40, 41**).

Strain X-1612

The crowning event in the wartime effort to secure improved strains of penicillin-producing molds was the emergence of "super-strain"

X–1612, which was obtained through mutagenic (X-ray) treatment of conidia of *NRRL 1951·B25*. As Raper (**39**) has pointed out, "The production of this culture should be regarded as a joint endeavor. The stock was supplied by the Fermentation Division, Northern Regional Research Laboratory; the irradiation was performed by Dr. Demerec and associates at the Carnegie Institution, in Cold Spring Harbor; the initial and indicative production tests were made at the University of Minnesota, by Drs. Christensen and Ehrlich; and the real magnitude of its superiority was demonstrated, at the University of Wisconsin, by Professors Peterson and Johnson in 80-gallon fermenters." This "super strain" was shown to yield approximately twice as much penicillin as its parent, and during 1945 it became the principal race used in commercial production of the drug. Cultural characteristics and the metabolism of this variant are described in the literature (**5**, **17**, **28**, **30**, **32**, **33**, **39**, **42**, **52**, **53**).

In relation to the present communication, special interest centers on strain *X–1612*, since it is the immediate ancestor of the entire "Wisconsin Series" of *P. chrysogenum* strains. Strain *X–1612* first came into the hands of the writers early in the fall of 1944, before its true merit had been established. It was nevertheless almost immediately adopted by the authors as the base stock to be used in a new strain development program being launched at that time, for, shortly after being received, it was tested in shake-flasks in the writers' laboratory along with several strains which had been rated among the best available at that date, and it gave better penicillin yields than any of the competing cultures.

CULTURAL METHODS

The principal solid substrate used for the cultivation of the fungus in the strain development program carried out was honey-peptone agar, a Sabouraud-type medium consisting of 6% honey, 1% Difco bacto-peptone, and 2% agar. To minimize caramelization of the sugar in the preparation of this substrate, the agar is dispersed in the water before the other ingredients are added. The autoclaved mixture has, without adjustment, a pH favorable to fungal growth and inhibitory to most bacteria. This medium was used to prepare tube and bottle slants and Petri plates.

Conidial suspensions, as needed, were made up over agar slant cultures of appropriate age—mainly cultures which had been incubated for 7–10 days at 24–25° C. Sterile distilled water, sometimes with a trace of wetting agent, was introduced into the culture vessel to fill the latter

approximately half full. Agitation of the liquid, accompanied when necessary by gentle scraping of the surface of the culture with a transfer loop or serological pipette, served to bring the spores into suspension. Sterile pipettes and water blanks were employed to make dilutions. When suspensions of a particular spore concentration were required, counts were made with a haemacytometer and adjustment of the spore load accomplished by dilution procedures. In some instances spore suspensions were passed through sterile Whatman No. 2 filter paper to remove mycelial fragments and eliminate spore clumps.

For plating, a standardized procedure has been followed, although spore suspensions of various histories have been handled. Plates are poured in advance and allowed to solidify on a leveled table top in a culture room. Then onto the surface of each plate of agar one milliliter of a suitably diluted spore suspension is introduced with a sterile pipette. The plate is rotated to spread the suspension over the agar, after which it is returned to its original position and left to incubate at room temperature.

In a limited number of instances, individual sporelings were isolated through a modification of the single-spore isolation technique described by Thom and Raper (**55**). In this procedure, a diluted spore suspension is spread onto hard (4%) cleared agar containing 0.5% sucrose as the only nutrient. After approximately 15–20 hours (the time varying with the strain) at room temperature, germination has usually progressed to the point where isolation can be begun. The operator, working under a high-powered stereoscopic microscope, then proceeds to remove selected sporelings together with a small square of the underlying agar, utilizing a microscalpel fashioned from a fine sewing needle which has been fused into a glass rod and ground flat on a carborundum stone. This tool is sterilized by dipping it into a vial of alcohol and then quickly running it through a flame. The same equipment has been employed to remove and transfer hyphal tips from the margins of colonies.

A long succession of populations, each arbitrarily limited in size to about 250 isolates, has been studied. Almost invariably these populations have been obtained by seeding plates with a dilute spore suspension and then transferring hyphal tips from the margin of each young colony after about three days incubation. For study of the cultural characteristics of the individuals in such a population, hyphal tip transfers are made to honey-peptone agar plates containing measured amounts of the medium. Four such transfers, evenly spaced, are made onto the agar surface of each culture dish (FIG. 1, B), and the transfers are numbered. The 60–65 plates required to accommodate a given population are then

incubated at 24–25° C for a week to ten days. At the same time that the hyphal tip transfers are made to the agar plates, duplicate transfers are also made to correspondingly numbered tube slants. After a suitable incubation period, a second tube slant is seeded from each original tube culture by mass-spore transfer. This subculture, which matures rapidly

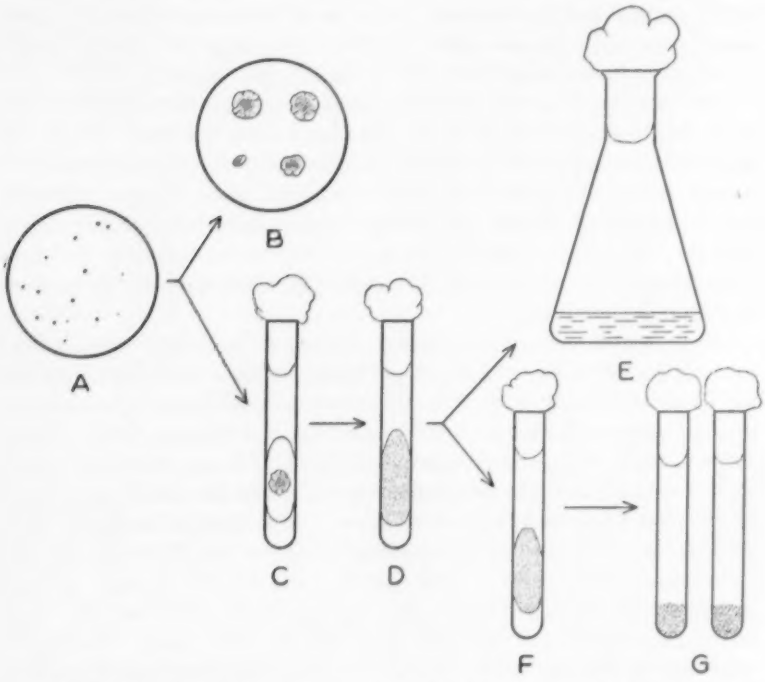

FIG. 1. Diagram illustrating sequence of cultures prepared in processing a population. A. Isolation plate. B. One of a group of plates prepared for study of the cultural characteristics of the isolates. C. Original tube culture of a given isolate. D. Sub-culture No. 1, inoculated by mass-spore transfer from the original tube culture. E. Fermentation flask (for screening test). F. Sub-culture No. 2. G. Master soil preparations.

because of the heavy seeding, furnishes inoculum for an antibiotic screening test. A conidial suspension is made up over this culture and one ml of it is used to seed the broth in a fermentation flask. A loopful of the same suspension is used as inoculum to start tube subculture No. 2. This second sub-culture is eventually either discarded or is employed to set up soil preparations, depending upon the quality of the

isolate, as revealed by the outcome of the fermentaion test, etc. For each isolate selected for further study, two duplicate soil preparations are set up from the source indicated above. This pair of soil preparations constitute the "master" cultures for the given isolate and are carefully preserved in the permanent collection. All further operations involving any particular isolate are based upon these master stocks. This basic sequence of cultures, ending with the master stocks, is shown schematically in FIG. 1.

In setting up the soil preparations a modification of the technique described by Greene and Fred (**19**) has been followed. This involves pipetting 1 ml of a heavy conidial suspension of a given race onto 5 gms of dry, thoroughly sterilized soil (a mixture of equal parts of quartz sand and well-screened and pulverized garden loam) in a culture tube stoppered with a gauze-wrapped cotton plug. The inoculated soil tube is then allowed to dry out slowly at room temperature, covered by a porous paper cap. Once prepared and dried, the soil stocks require no further attention. In the writers' laboratories they are not refrigerated and they are given no special care except to keep them protected from dust. Such soil stocks are not only very easy to set up but serve to maintain the various strains in an unchanging condition over a long period of time. Cultures prepared in this laboratory as long ago as 1944 are still viable and apparently have undergone no degenerative changes. To recover the fungus in active condition it is only necessary to remove aseptically a small quantity of the soil and dust it over the surface of a fresh agar slant. For this purpose a tiny dipper, fashioned from the end of a hammered inoculating needle, is convenient. The same soil tube can be used repeatedly.

Secondary soil preparations in lots of thirty to fifty tubes have been set up for some isolates, and to provide the necessary amount of spore suspension in these cases large bottle slants seeded with soil from the master stocks have been used. Since this laboratory has attempted to supply stock cultures of certain key strains to all penicillin producers and research laboratories requesting them, several successive lots of this magnitude have had to be prepared to meet the demand in a number of instances. In setting up these lots, the unvarying procedure has been to go back to one of the original "master" soil preparations for inoculum. Furthermore, sample tubes from each new lot have been checked against the master culture by fermentation test. These practices have resulted in a high degree of uniformity among successively prepared lots of stock cultures of a given strain.

MUTAGENIC TREATMENTS

Although a substantial amount of spontaneous variation has been encountered in the *P. chrysogenum* lines involved in this study, it has been found that through mutagenic treatments of spores the amount of variation can be increased. Furthermore, through mutagenic treatments there are obtained types of variants which either do not occur spontaneously at all or which appear so rarely that none of them has yet been detected in this laboratory (**50**). Two types of mutagenic agents have been employed in the program out of which the Wisconsin family of strains has emerged: ultraviolet radiation and the nitrogen mustard, methyl-bis(β-chloroethyl)amine.

The irradiation treatments have been carried out in a special vessel, similar to one used by Duggar and Hollaender (**13**), of the design illustrated in FIG. 2. This vessel is made of glass but has quartz windows. Through the side arm a spore suspension to be treated is introduced and treated samples can be removed. A sterile stirring rod, driven by a motor, fits through the vertical upper arm and its tip rotates to one side of the center of the main chamber where it effectively circulates the suspension but is not in the path of the UV rays entering the window. Prior to an irradiation treatment the vessel is sterilized by flushing it with 70% ethyl alcohol followed by rinsing with sterile distilled water. While a treatment is in progress the side arm is capped with a glass vial and a glass baffle around the top of the stirring device prevents entrance of contaminants from above.

A 1000-watt General Electric AH–6 water-cooled, high pressure mercury vapor lamp has been used to furnish ultraviolet radiation for the treatments. The lamp is housed in a radiation-tight box, and the image of the arc is focused on the front slit of a Bausch and Lomb quartz monochromator by means of a quartz lens affixed to an opening in the lamp house. Radiation from the rear slit of the monochromator passes through a quartz lens, through the irradiation vessel, and on to the receiver of a thermopile. The latter as part of a thermopile-galvanometer system was standardized for absolute energy measurements. For the treatments used in the phases of the project in question here, ultraviolet radiation of 2750Å and 2534–37Å has been exclusively employed.

When a treatment is to be carried out, a spore suspension is prepared and adjusted to a concentration of about 100,000 conidia/ml. With a sterile pipette ten ml of this suspension are introduced into the irradiation vessel. Just before the irradiation is begun, a 0.2 ml control sample is removed and placed in a 100 ml sterile water blank. Additional 0.2

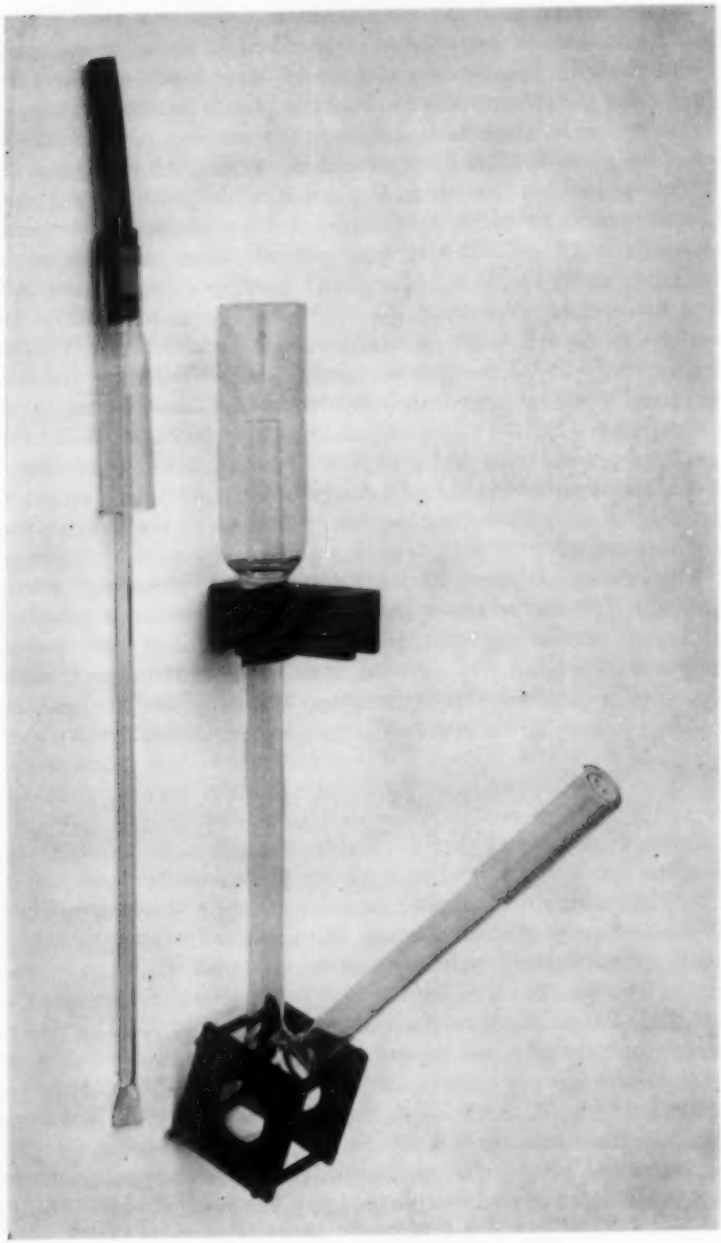

FIG. 2. Irradiation vessel. Capacity, 10 ml.

ml samples are taken at five-minute intervals throughout the progress of the treatment, and these are likewise placed in 100 ml water blanks. The irradiation is usually continued for 1 hour. A few milliliters from each diluted sample are then immediately plated out to determine the number of spores which have survived the treatment. The remainder, representing the bulk of each sample, is refrigerated for a few days until the desired information on killing is available from the test plates. Then the remainder of the one sample showing the desired percentage survival is plated out. During the early part of the program, samples which exhibited up to 99.4% killing were used; in all of the more recent work, however, samples showing a survival of approximately 25% have been chosen, since it had been found that with strain NRRL *1951* this survival level yielded the greatest number of morphological variants—a situation in general agreement with what has been described in the case of *P. notatum* (**22**, **49**) and certain other fungi (**8**, **23**, **24**, **25**). From the plates prepared, an irradiation-survivor population is obtained in the fashion described in the preceding section. This is then handled according to the plan worked out for processing all populations, whatever their origin.

The methods followed in treating spore suspensions with nitrogen mustard have been described elsewhere (**45**). Because with nitrogen mustard treatments the percentage of morphological variants increases more or less progressively with the duration of the treatment, samples containing a relatively low percentage of viable conidia have been employed to provide the survivor populations in this case.

FERMENTATION TESTS

In view of the fact that the ability of a given isolate to produce penicillin has been regarded as a matter of major importance and has, indeed, often been the principal basis for selection of key forms in the Wisconsin family of strains, much effort has naturally gone into the task of evaluating the productivity of the cultures.

The fermentations have been carried out in 500 ml Erlenmeyer flasks containing 100 ml of a corn steep-lactose liquid medium. This medium, throughout the study, has consisted of the following basic ingredients: distilled water, corn steep solids 2.0%, crude lactose 4.0%, $NaNO_3$ 0.3%, KH_2PO_4 0.05%, $MgSO_4$ 0.025%, and $CaCO_3$ 0.3 to 0.4%. The amount of calcium carbonate used was varied as indicated to bring the pH of the medium to 5.3–5.6 after sterilization. In essentially all of the tests carried out since 1947 an antifoam agent has been employed, 3 drops

of Dow Corning "Antifoam A" emulsion (silicone) being introduced into each flask prior to autoclaving. Also beginning about 1947 a penicillin precursor, β-phenylethylamine (Monsanto), neutralized with acetic acid, has been added, usually at a level of 0.2% or 0.25%. This adjuvant likewise has been introduced into each flask separately prior to sterilization of the broth. The flasks are plugged with cotton, sterilized for 15 minutes at 15 lbs steam pressure, and, when cool, are seeded with spores of the isolate to be tested. One ml of an undiluted conidial suspension, made up over a fresh agar slant culture (usually 7–10 days old), is used as inoculum. The seeded flasks are then placed on a reciprocating shaker operating at 90–95 cycles per minute with a 4-inch stroke, in a fermentation room thermostatically controlled at 24–25° C. At appropriate intervals broth samples are removed with a sterile pipette, diluted with phosphate buffer, and assayed by the cylinder-plate method of Schmidt and Moyer (**47**).

One of the chief aims of the fermentation tests carried out on a given population was to detect whether there might be in this population variants possessing increased capacity to produce the antibiotic. Since accurate evaluation of the ability of a culture to produce penicillin is not simply accomplished, the procedure adopted may be considered as something of a compromise. Although it was not as thorough as the writers would have liked, it was as intensive as could be arranged for the handling of large numbers of cultures. That the selection program, as carried out, was in some measure effective is attested by the fact that it has yielded improved strains suitable for industrial use.

The initial step in conducting the fermentation studies on a given population has been to run a "screening" test. In this test a single flask is used for each of the approximately 250 isolates to be evaluated, and assays are run at six and seven days. Several flasks inoculated with the parent strain and usually also others inoculated with older strains of known potency are included as controls in each fermentation run. When the screening test is completed, about a dozen of the isolates which have given the highest yields of the antibiotic are selected. A repeat test on these isolates is then set up on a larger scale. Three or four flasks of medium are inoculated with each strain in this trial. Yields are averaged and again those isolates giving the best performance are picked out. In a third test, three or four "finalists" may be put in competition, with up to ten flasks being used for each strain. On the basis of performance in this test a single culture is selected to be used in starting a new "generation"—with or without intervening mutagenic treatment. However, it is not invariably the isolate which comes out best in these

fermentation tests that is chosen to form the basis for further operations, since cultural characteristics and other traits may also enter into the picture when the final selection is made. If, as occasionally happened, no isolate in the population gave yields better than the parental form, the usual procedure was, nevertheless, to take what appeared to be one of the best individuals in the population and carry on with it.

Certain key strains emerging in the course of the work have been subjected to additional tests and study in the writers' laboratories, and a few of them have been submitted to the Fermentation Laboratories of the University of Wisconsin Department of Biochemistry for more critical evaluation. Here tests have commonly been run in flasks on a rotary shaker, in 30-liter stirred jars, and occasionally in 80-gallon tanks; the performance of the strains on various media, under varying amounts of aeration, at various precursor levels, etc. has also often been studied by the biochemists. A detailed analysis of chemical changes occurring in the medium as the fermentation progressed has usually been made (**1**, **6**, **17**, **26**, **29**, **36**, **53**).

STRAIN WIS. Q176

Origin and Productivity

As has been pointed out above, the first significantly-improved strain to emerge in the course of work conducted in the writers' antibiotics research program was obtained in 1945. This variant, designated as Wis. *Q176*, begins the "Wisconsin Family" of strains.

During 1944–45 a series of irradiations had been carried out on a variety of *P. notatum* and *P. chrysogenum* lines. Irradiation "*Q*" was the second in which spores of strain *X–1612* were treated, and it was colony number *176* in the irradiation-survivor population obtained following this treatment which yielded the notable strain in question.

For the sake of precision, it should be stated here that a special procedure was involved in preparing the *X–1612* stocks employed in these irradiation experiments. Because the writers wished to be sure that they were dealing with a pure line, and because at that time only scanty information was available concerning the history of the "*X–1612*" culture which had been received from the University of Minnesota, a single spore was isolated to start the *X–1612* stocks for this laboratory. However, in view of the fact that this single-spore line agreed closely with the parental culture in growth characteristics and fermentation pattern, it is believed that little significance need be attached to this single-sporing step in tracing the genealogy of the Wisconsin Family.

As has already been mentioned, it was the practice in the early irradiation treatments carried out in this laboratory to prepare the survivor populations from suspensions in which a very large portion of the conidia had been killed. In the suspension used from Irradiation Q less than 1% of the spores were viable.

In the screening test carried out on survivor population Q, many of the individuals yielded appreciably less penicillin than did the *X–1612* control culture, many showed yields of approximately the same magnitude as those from the parent, but for individual *176* the inhibition zone on the assay plate was much larger than any of the other zones at both the six and the seven day assay. The performance of this variant in the larger-scale repeat tests was equally good, and it was soon established that an outstanding new strain had been secured. Tested in 80-gallon fermentors in the University of Wisconsin Biochemistry Department, the new strain gave yields of over 900 O.U./ml in contrast to about 500 O.U./ml obtained with strain *X–1612* run under the same conditions (**4**, **17**).

Released late in 1945, strain Wis. *Q176* was widely sought by commercial producers of penicillin, perhaps as much for use as a "breeding stock" in their own strain development programs as for use in their production plants. Microbiologists at universities, etc. also chose this variant as a base stock for work along similar lines and for a variety of fundamental studies (**2**, **3**, **9**, **14**, **37**, **38**, **43**, **46**, **48**, **56**, **57**, **58**). The strain was likewise employed in detailed chemical studies on the fermentation process (**6**, **7**, **16**, **18**, **20**, **26**, **27**, **34**). Over 150 soil preparations of strain *Q176* have been distributed by the writers in response to requests from industrial laboratories, university laboratories, microbiological institutes, culture collection centers, public health organizations etc. in nineteen countries.

What at first appeared to be a weakness of the strain, namely, the fact that it tended to produce a high proportion of penicillin K, was soon overcome when it was discovered that simply by adding to the fermentation broth a suitable precursor (phenylacetic acid or various derivatives of this compound) production could be shifted over almost completely to the desired penicillin G (**1**, **6**, **7**, **20**, **21**). In addition, the use of a precursor was found to increase the total yield of the antibiotics. In contrast to some of the later-derived strains of the Wisconsin Series, which are apparently exacting in their requirements regarding fermentation conditions, strain *Q176* appears to be adapted to perform well under a wide range of circumstances. To judge by reports received, yields surpassing those from *X–1612* seem to have been almost universally

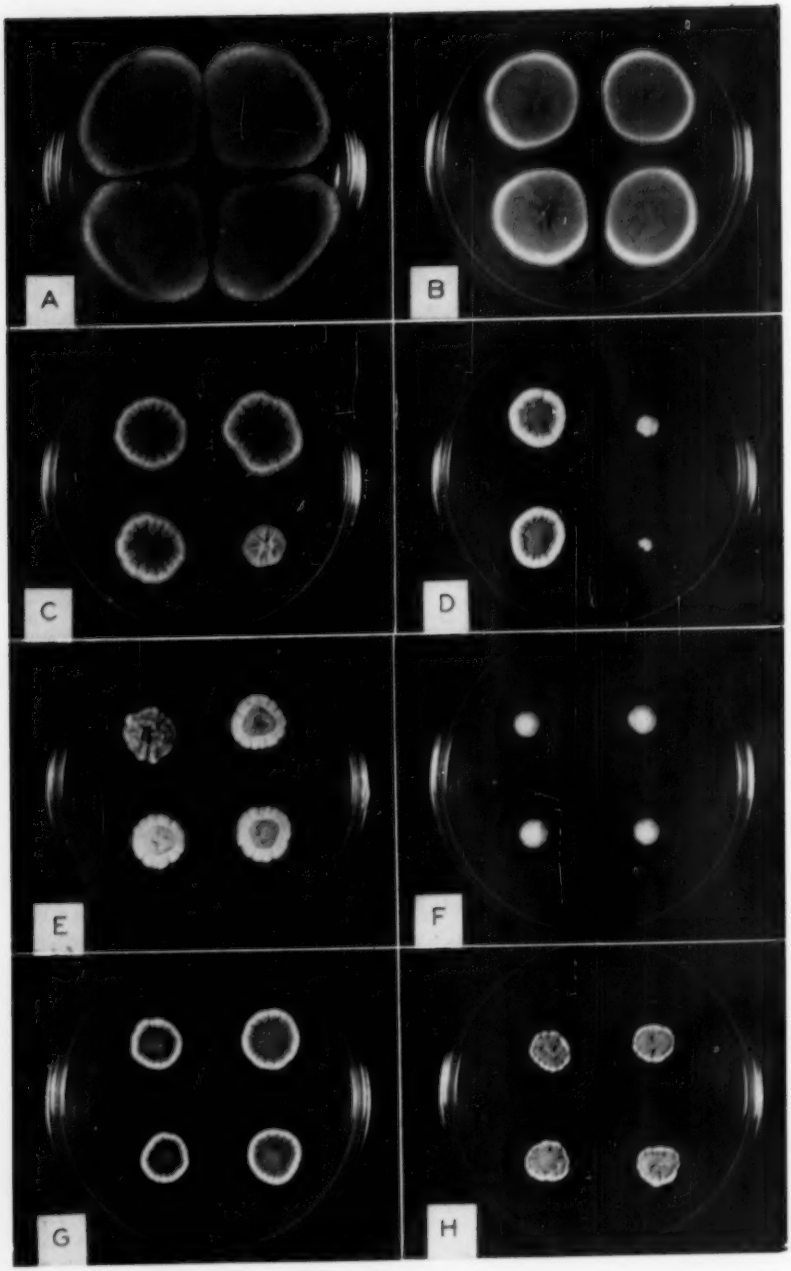

FIG. 3.

obtained with this variant, and it became widely known as a standard culture for penicillin production (**29**).

Cultural Characteristics

Mass spore transfer cultures of Wis. *Q176* on honey-peptone agar resemble those of *X–1612* but mature somewhat more slowly and sporulate less heavily. There is, indeed, progressive reduction in rate of growth and amount of sporulation following through the entire line of descent from *NRRL–1951* to Wis. *Q176*. This is best observed when a few dozen separate sporelings of each strain are transferred to individual honey-peptone agar plates and the development of the monosporous colonies is studied. After six days incubation at 24–25° C, colonies of *NRRL–1951* have reached an average diameter of about 40 mm, those of *NRRL–1951·B25* approximately 30 mm, and those of *X–1612* about 25 mm, while the average diameter of the *Q176* colonies is less than 20 mm at this age. Colonies of *NRRL–1951* have a flat, spreading growth on honey-peptose agar; in *NRRL–1951·B25* and *X–1612* there is slight radial folding, and such folding becomes very conspicuous in *Q176* (FIG. 3, C). As convolutions increase, the colony outline also tends to become irregular. Conidia are produced in great abundance in the wild-type ancestor, but sporulation is substantially diminished as one moves through the series, and the spores are slower to appear. Raper and Alexander (**41**) have recorded the occurrence of abnormal penicilli in strain *1951·B25*. Irregularities in penicillus structure are also encountered in *X–1612* and *Q176*. Furthermore, in strain *Q176* there are marked abnormalities in some of the vegetative hyphae. Campbell (**10**) and Churchill (**11**) have made a study of the mycelium and reproductive structures in this strain, and some of the microscopic peculiarities have been illustrated in a recent communication from this laboratory (**50**).

The preparation of populations from untreated spores of Wis. *Q176* first brought to light a remarkable pattern of spontaneous variation which has since been found to extend throughout most of the Wisconsin family of strains and which has been designated "the population pattern phenomenon." Some features of the situation involved here have already been outlined by the writers (**50**) and the "phenomenon" will be dealt

FIG. 3. Plate cultures showing 7-day old colonies from populations of various strains: A. Strain *NRRL 1951;* B. Strain *X–1612;* C. Strain Wis. *Q176;* D. Strain Wis. *48-701;* E. Strain Wis. *49–133;* F. Strain Wis. *51–20;* G. Strain Wis. *51–20A;* H. Strain Wis. *53–844*. Each plate contained 25 ml honey-peptone agar. Inoculation was by hyphal-tip transfers and incubation was at 24–25° C.

with in detail in a later publication. For the purposes of the present report, only those items which bear directly upon the origin and characteristics of the various key Wisconsin strains need be considered.

When sizable populations of strain *Q176* are set up as previously described (see Cultural Methods section above), or if *Q176* sporelings are isolated at random and transferred four to a plate, it is found that the colonies present after 7–10 days incubation are not all alike, as one might expect they would be. Instead, it can be observed that there are mainly five fairly distinct types of colonies present; and a further remarkable fact is that whenever *Q176* populations are prepared, these five colony types always appear in approximately the same proportions. One kind, designated as the "U-type" (usual type), predominates, accounting for roughly 65–75% of the population. "D"- and "C"-type colonies are next most abundant, while "B"- and "A"-types are always

TABLE I

A TYPICAL POPULATION PATTERN IN STRAIN WIS. Q176

Colony type	Av. diameter of colony in mm.*	Occurrence in population per cent
U	31.3	65.0
D	23.4	20.0
C	14.7	8.5
B	12.0	2.5
A	12.5	4.0

* Colonies ten days old, grown singly in Petri dishes on 25 ml honey-peptone agar, at an incubation temperature of 24–25° C.

present in very small numbers. The proportion of colony types found in one typical *Q176* population is recorded in TABLE I. This table further indicates the general difference in colony size which is encountered. The mycelial mats of "C"- and "B"-type colonies are typically strongly raised. Sporulation diminishes progressively from the U-type through the D-, C-, and B-types. The small B-type colonies usually have only a faint tinge of green near the center, where most of the scanty spore production is concentrated. The A-type colony departs most strongly from the usual type. It forms no conidia at all, and it has a distinctive flesh color; the mat is thick and compact but tends to be translucent and may have a water-soaked appearance. A- and B-type colonies show a strong tendency to sector, with the sectors growing much more rapidly than the parent and sporulating. Hyphal-tip transfers from such sectors usually yield typical U-type colonies.

From the situation just related, it follows that one cannot describe the cultural characteristics of a strain like *Q176* in simple terms. Since

a spore suspension or soil preparation of this variant contains conidia of five or more definitely different potentials, it also follows that the usual concept of a strain must be modified in applying the term to the entity at hand. Strain *Q176* is not to be thought of as a homogeneous entity but rather as a balanced mixture of several components, each with its own distinctive pattern of behavior. *Q176* may be defined, in part, in terms of its population pattern; and, because of the predominance of the U-type colony, it may properly be referred to as a U-type strain.

STRAIN BL3–DIO AND THE BEGINNING OF THE WISCONSIN PIGMENTLESS SERIES

About the time that strain *Q176* was obtained, it began to appear that in addition to increased ability to produce penicillin there were other traits to be encountered among variants which might make a stock more desirable for commercial use. One such trait concerned the matter of pigment secretion by the fungus.

As is well known, most isolates of *P. chrysogenum* obtained from nature tend to produce relatively large quantities of water-soluble yellow pigment. This passes out into the broth in a liquid culture, and in cultures on Czapek's agar it not only diffuses into the substrate but colors the droplets of liquid which characteristically accumulate over the surface of the colony. Indeed, so consistently is this trait encountered that Thom, in naming the species, chose the specific epithet *"chrysogenum"* as a descriptive term. The variant strains *NRRL–1951, B25, X–1612,* and Wis. *Q176,* like their wild-type ancestor, *NRRL–1951,* produce yellow pigment in abundance, and commercial producers using such strains were forced to remove the pigment if they desired to market a white product. By 1946 it was general practice to completely remove the pigment although the necessary extraction procedures usually resulted in loss of some of the antibiotic. In earlier years the pigment was not all removed, and the penicillin of commerce at that time had a golden color.

It was suggested to the writers that a mutant strain which did not form any pigment in the medium would be industrially desirable, particularly if such a strain also had potency in penicillin production equal to that of Wis. *Q176.* That it might not be easy to secure such a strain, however, was indicated by certain observations which had been made in this laboratory, not only in conection with the improved *P. chrysogenum* lines being studied at that time but also in connection with the various *P. notatum* strains worked with previously: (1) even in populations grown from conidia surviving mutagenic treatment almost all the

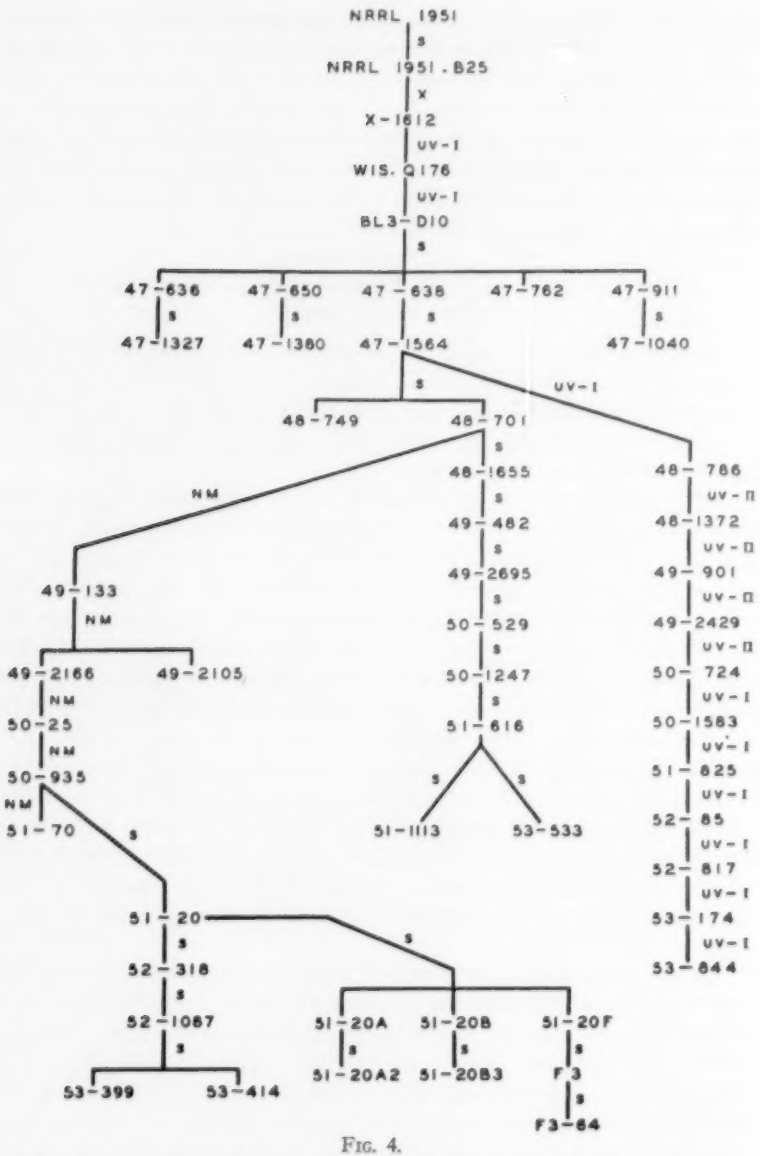

FIG. 4.

FIG. 4. Genealogy of the Wisconsin Family of strains of *Penicillium chrysogenum*. S indicates selection without mutagenic treatment; X indicates selection following X-irradiation; UV-I indicates selection following 2750Å UV-irradiation; UV-II indicates selection following 2534-37Å UV-irradiation; NM indicates selection following nitrogen mustard treatment.

isolates produced pigment in some amount, (2) isolates secreting a reduced amount of pigment almost invariably were also found to yield reduced amounts of the antibiotic, and (3) the rare isolates which seemed to form no yellow pigment at all were variants which had been severely damaged by radiation.

Nevertheless, during the spring of 1947, in studying populations grown from spores which had been subjected to various amounts of UV radiation, a very promising completely pigmentless strain, *BL3–D10*, was encountered. Isolate *BL3–D10* was found in a population grown from *Q176* conidia which had received a very low dosage of radiation. The type of population involved has been termed an "ultraviolet-stimulated population" (**51**), since conidial suspensions of *Q176* so treated actually yield more colonies than do the unirradiated controls. Although *BL3–D10* produced only about 75% as much penicillin as *Q176* in the fermentation tests conducted, this was by far the highest yield that the writers had encountered in any variant which formed no yellow pigment. Furthermore, this was a vigorous race compared with the only other completely pigmentless variants that had been seen heretofore in this laboratory. It was therefore decided to utilize this as a "breeding stock" in attempting to obtain additional pigmentless variants which might be better producers of penicillin than *BL3–D10* itself appeared to be. This proved to be a wise decision, for among the derivatives eventually obtained from this strain there were many which surpassed *Q176* in ability to produce the antibiotic, while maintaining the pigmentless quality; and some had further advantages as well. As a matter of fact, all the remaining members of the Wisconsin family of *P. chrysogenum* strains are descendants of strain *BL3–D10* and belong to the Wisconsin "pigmentless series." Once arrived at, pigmentlessness proved to be an enduring character and has been found to persist almost without exception through all the many thousands of descendants of *BL3–D10* which have been studied.

As indicated in the genealogy chart (FIG. 4), the next step in the development of the Wisconsin pigmentless series involved a program of selection without further mutagenic treatment. During 1947 a series of populations were prepared and over 2000 cultures were screened. From among these, nine key strains were selected, and four of them, *47–911*, *47–1040*, *47–1380*, and *47–1564*, in repeated tests gave yields at least as good as those obtained from *Q176* under the fermentation conditions employed. On August 1, 1948, all four strains were made available for general use. Because further tests appeared to indicate that strain *47–1564* was probably slightly superior to the other three, this

strain was chosen as a parental stock from which to prepare additional untreated populations in 1948. Two more key strains, *48–701* and *48–749*, were selected when the testing work had been completed, and the release of these strains followed soon afterwards. Yields obtained with these strains in this laboratory have averaged 15%–25% above those secured with the parent, *47–1564*, when the latter was used as a control in the tests. Strain *48–701* has commonly outperformed *48–749* by small margins in fermentations conducted on this campus, and the two strains have been shown to have a somewhat different population pattern (TABLE II); in most respects, however, they are very similar.

In contrast with strain *Q176*, all of the pigmentless strains which have been mentioned above are D-type races. That is, the D-type colony predominates in populations grown from untreated spores; and the U-type

TABLE II

TYPICAL POPULATION PATTERNS IN KEY STRAINS OF THE WISCONSIN PIGMENTLESS SERIES COMPARED WITH THE PATTERN IN Q176

Colony type	Percent						
	Strain Q176	Strain 47–911	Strain 47–1564	Strain 48–701	Strain 48–749	Strain 49–133	Strain 51–20
A	3.7	3.6	7.1	7.6	0.4	3.6	0.5
B	2.6	5.0	2.9	8.4	1.3	0.7	5.4
C	8.5	13.3	5.4	9.3	7.6	4.3	94.1
D	20.2	76.3	84.2	74.7	90.8	91.3	0.0
U	64.8	0.0	0.0	0.0	0.0	0.0	0.0
All others	0.4	1.8	0.4	0.0	0.0	0.0	0.0

colony, which is so characteristic of *Q176*, has almost completely disappeared. Typical population patterns in four of the pigmentless strains under discussion are included in TABLE II. It must be pointed out, however, that the D-type colonies of strains *48–701* and *48–749* differ appreciably from those encountered in strains of the "*47*" series. Roegner (**44**) has distinguished several sub-types in the "D-group" and points out that most of the D-type colonies of strains *48–701* and *48–749* fall in subgroup IV, whereas in *47–1564*, their parent, they are predominantly of the "D–II" type. The chief distinguishing feature of the D–IV colony in contrast with other sub-types is a pale color, resulting apparently from the presence of short sterile aërial hyphae which are intermingled with the conidiophores and in part overgrow them. Because all of the Wisconsin pigmentless strains thus far considered have D-type population patterns, it follows that in all of them the growth on agar is slower and sporulation is somewhat less than in strain *Q176*. The

decreased rate of growth of variants *47–1564* and *48–701* is evident in TABLE III. Interestingly enough, however, the mycelial development in aerated liquid cultures on corn steep-lactose medium appears to be about as rapid as it is in the case of older strains in the family tree.

The various pigmentless strains differ from the grandparental strain *Q176* and to some extent from one another in a number of additional features. Of special interest is the fact that the pigmentless strains, in general, seem to utilize precursor more efficiently, tend to have lower oxygen requirements, and tend to oxidize lactose more rapidly (**29**). Especially rapid oxidation of lactose, with no initial lag phase in the utilization of this sugar, was reported in the case of strain *48–749* (**1**).

TABLE III

COLONY SIZE IN FOUR WISCONSIN VARIANTS AND IN THREE ANCESTRAL STRAINS *

Strain	Number of colonies		Average diameter (mm.)			
			7 Days		9 Days	
	Honey-peptone agar	Czapek-Dox agar	Honey-peptone	Czapek-Dox	Honey-peptone	Czapek-Dox
NRRL *1951*	49	11	53.6	35.3	—	—
NRRL *1951.B25*	49	11	39.3	24.9	49.8	30.7
X-1612	44	10	25.2	24.1	32.6	31.3
Wis. *Q176*	34	7	21.0	19.0	26.5	24.7
Wis. *47–1564*	42	8	15.9	12.9	19.8	16.9
Wis. *48–701*	25	7	15.0	14.0	18.5	18.0
Wis. *49–133*	43	9	14.0	11.3	18.8	16.0

* Colonies grown at 24–25° C in Petri dishes containing 25 ml medium. Each plate was inoculated with one 1-day-old sporeling chosen at random.

In addition to lack of pigment, there are a number of other features about some of the pigmentless strains which have made them attractive to certain commercial producers of the drug.

A THREE-WAY SELECTION PROGRAM IN THE WISCONSIN PIGMENTLESS SERIES

Results obtained in studies carried out on the mutagenic effects of nitrogen mustard on *P. notatum*, strain *NRRL 832* (**49**) were primarily responsible for the adoption, in 1948–49, of a new scheme of operations in carrying forward the strain development program in the Wisconsin Pigmentless Series. Beginning at that time, three separate lines of descent were followed, as indicated by the trifurcation in the family tree (FIG. 4). Since one of the principal aims involved in the new plan

was to determine the effects of repeated mutagenic treatments over a long series of generations, the approach was academic. However, a number of outstanding strains have emerged in the course of the work.

Selection without Mutagenic Treatment

The control branch of the three-way line of descent was anchored on strain *48–701*, although, as inspection of the genealogy chart will make clear, it actually extends back to strain *BL3–D10* through strains *47–1564* and *47–638*. Each step in the progression has involved the preparation of a population from untreated spores and then carrying through the cultural and fermentation tests upon which were based the selection of the key strain which was to serve as the parent for the next generation. In this manner the selections have been carried on slowly through a total of eight generations beyond Wis. *48–701*. Although no spectacular changes in ability to produce the antibiotic have occurred at any point, a gradual rise in productivity can be traced up through strain *50–1247* —beyond which no further improvement could be effected.

Repeated Ultraviolet Treatments

In the second line of descent, a similar routine of testing and selection has been followed, but in this case each population dealt with was a survivor population prepared from a spore suspension which had been subjected to sufficient ultraviolet radiation to kill approximately three-fourths of the spores. Ultraviolet of two different wavelengths, 2750Å and 2534–37Å, has been employed in the treatments. A survivor population grown from irradiated spores of strain *47–1564* provided the material upon which operations were begun in this portion of the three-way program, and the "ultraviolet line" has been continued through twelve generations (FIG. 4). As in the case of the simple selection (control) line, no strains in the UV series have become widely known, since at the time of their emergence they were eclipsed by still better strains from the "nitrogen mustard branch" of the family tree. Nevertheless, there have been substantial gains in ability to produce the antibiotic. Through the first few generations there was no significant improvement over the ancestral form *47–1564*. However, gains of varying magnitude have been recorded at various points along the line of descent between the fourth and twelfth generations. Thus the key strains in the latter part of this series are, in general, very good producers of penicillin. Not only are they clearly superior to *47–1564*, from which they sprang, but also they are better than any of the strains in the parallel control series

in which no irradiation was involved. All key strains obtained up to this time in the UV series are of the D- or C–D-type (FIG. 3, H).

The Nitrogen Mustard Line

Although it was originally intended that the third line of descent in the three-way selection program should exactly parallel the other two, differing only in that repeated nitrogen mustard treatments were to be employed in obtaining the populations from which key strains would be selected, complications were introduced by the occurrence of two noteworthy strains falling outside of the regular sequence, as will be explained below.

The nitrogen mustard line is of special interest because the best strains of *P. chrysogenum* to be developed in this program have emerged here. A spore suspension of strain *48–701* which had been treated with the mutagenic chemical provided the initial survivor population in this series, and the high quality of one individual in this population is primarily responsible for the general superiority of the nitrogen mustard branch of the family tree. The key strain selected from this first group of survivors was Wis. *49–133* (FIG. 3, E and TABLE II), which embodies the greatest single improvement effected in the Wisconsin Pigmentless Series. Penicillin yields 50% to 100% above those obtained from the parent, *48–701,* have commonly been recorded for strain *49–133* when the two variants have been included in the same fermentation run. In early tests the superiority of the nitrogen mustard-derived variant over the parental and grandparental strains was repeatedly exhibited and was most marked at a high precursor level (TABLE IV).

In the second generation, Wis. *49–2105* was the highest-yielding strain encountered in the survivor population; this, however, could not be selected as the key strain to continue the main line of descent because of an interesting defect: it produced a pigment in the medium. The appearance of a pigment-producing variant in a long-established pigmentless stock was of course an item of considerable academic interest, particularly since this was the only strain of that character to be encountered among the thousands of descendants of *BL3–D10* studied in this laboratory. However, since the pigment formed by strain *49–2105* is reddish brown and clearly different from the golden yellow pigment characteristic of the species, it is evident that the phenomenon in question cannot be interpreted as reversion.

Studies conducted in the University of Wisconsin Department of Biochemistry (1) have revealed that strains *49–133* and *49–2105* are

more efficient producers of penicillin than older members of the Wisconsin Pigmentless Series. In certain tests these nitrogen-mustard-induced variants were found to produce only about 50% as much mycelium as strains such as *47–1564* and *48–701*, yet gave higher penicillin titers. In the same studies exceptionally efficient utilization of the penicillin precursor, phenylacetic acid, by strain *49–133* was noted.

Between 1949 and 1951 five generations of nitrogen mustard treatment survivors were processed; and four key strains, in addition to

TABLE IV

PENICILLIN PRODUCTION IN SHAKE-FLASKS BY STRAIN WIS. Q176 AND CERTAIN OF ITS PIGMENTLESS DESCENDANTS *

Run no.	Concentration of precursor (β-phenylethyl-amine)	Strain	No. flasks	Av. yields (O.U./ml)	
				6 Days	7 Days
137	0.0%	*Q176*	3	293	298
		47–1564	3	291	362
		48–701	3	258	354
		49–133	3	218	435
137	0.1%	*Q176*	3	420	452
		47–1564	3	738	705
		48–701	3	823	870
		49–133	3	1060	1223
137	0.2%	*Q176*	3	310	402
		47–1564	3	505	695
		48–701	3	807	863
		49–133	3	1147	1290
155	0.25%	*Q176*	5	346	519
		48–701	5	930	933
		49–133	5	1472	1734

* Composition of the basal fermentation medium and other fermentation conditions are given in the text.

49–133, were selected in the main line of descent. Strains *49–2166* and *50–25* from the second and third generations showed no significant improvement over *49–133* in ability to produce the antibiotic. In the case of the fourth generation key strain, *50–935*, however, somewhat better yields were repeatedly secured. This strain is of further interest since it arose from a C-type colony and exhibits a C-type population pattern—the first such variant to be selected as a key strain in this program of studies. No further improvement in strain quality was effected through use of the mutagenic chemical.

In 1951 attention was diverted from the main line of descent in the nitrogen mustard series by the appearance of strain Wis. *51–20*, obtained

from a population prepared from untreated conidia of *50–935*. This strain arose from a small C-type colony and yields a population best characterized as a "weak C"- or "C–B"-type (TABLE II). This means that all of the colonies in the population exhibit very slow growth and weak sporulation on honey-peptone agar (FIG. 3, F). Mass-spore transfers yield extremely poorly sporulating agar slant cultures. However, in shake-flask fermentation tests conducted in this laboratory, penicillin yields averaging up to 2500 O.U./ml were recorded.

The one undesirable trait of strain *51–20* appeared to be its limited capacity to form spores. Since this feature made the strain difficult to work with in laboratory studies and because it appeared that such a defect might also impair its usefulness in industry, an attempt was made to obtain from it strains of better cultural quality which would still retain this variant's potency. The results of fundamental studies on the behavior of various kinds of colonies suggested that this might best be accomplished by allowing sectoring to occur. A population of *51–20* was accordingly prepared and incubated until sectors appeared on some of the colonies. As expected, these were mainly faster-growing and more heavily sporulating. There were thus obtained various sub-strains, designated as "*51–20A*," "*51–20B*," etc., each originating as a hyphal tip transfer from a different sector. The results of the fermentation tests were disappointing, however. In general, penicillin production was found to decline as sporulation and vigor were increased over that of the parental strain, *51–20*. Nevertheless, three of the variants were retained for further study: *51–20A*, *51–20B* and *51–20F*. The first of these was markedly improved over the parent in capacity to sporulate (FIG. 3, G) but far inferior to it in production of the antibiotic. Strain *51–20B* showed only slight improvement over *51–20* in sporulation but gave yields about on a par with it. *51–20F* was intermediate between the other two sub-strains in sporulation and in penicillin production. Single-spore isolates from these three sub-strains were next tested. All of the derivatives of *51–20A* gave inferior yields; some of the derivatives of *51–20B* equalled or slightly surpassed the yields obtained from strain *51–20* used as a control; while those from *51–20F* were again intermediate. Strain *51–20F3* (= Wis. *F3*) was selected as the best in the latter group, but yields as good as those from the parental form, *51–20*, were never obtained from it. Interestingly enough, results quite at variance with these were reported when certain of the strains were tested in the University of Wisconsin Department of Biochemistry under different fermentation conditions. Here high yields were obtained with strain *51–20F3* and *51–20A* but poorer yields with *51–20B*. Mainly

on the strength of the performance of strain *51–20F3* in these latter tests, a population was prepared from it by isolating single spores. The key strain selected from this population, *51–20F3–64* (= Wis. *F3–64*) has usually equalled or surpassed strain *51–20* in penicillin production tests conducted in this laboratory, and its capacity to sporulate is substantially better than that of the latter strain.

One additional attempt to improve the *51–20* stock has succeeded in some measure. Although the surest way to obtain increased vegetative

TABLE V

PENICILLIN PRODUCTION BY REPRESENTATIVE WISCONSIN STRAINS OF PENICILLIUM CHRYSOGENUM TESTED SIMULTANEOUSLY

Strain	Sporulation rating*	Average yield (O.U./ml)**	
		6 Days	7 Days
Q176	4.0	605	640
47–638	4.0	740	980
48–1564	4.0	930	1357
48–701	3.8	1107	1365
50–1247	4.0	1257	1506
48–1372	4.0	904	1343
53–844	2.6	1501	1846
49–133	2.2	1977	2230
49–2105	2.1	1798	2266
51–20	1.5	2140	2521
F–3	3.4	1767	2140
F3–64	3.1	2140	2493
53–414	2.5	1990	2580
53–399	3.1	2018	2658

* Scale 1.0 to 10.0: 1.0 signifies extremely weak sporulation, the cultures being almost white; 4.0 signifies "good" sporulation (Q176 level); 10.0 signifies very heavy, early sporulation such as is encountered in the wild-type ancestor. Figures given are averages of four separate ratings of 7-day-old honey-peptone agar slant cultures by two individuals.

** In each case, the yield given is the average of 12 flask cultures, 4 each from Runs 433, 435, and 442. In each of these runs the fermentation broth contained 0.25% β-phenylethylamine; other fermentation conditions as described in text.

and reproductive vigor in a C- or B-type race is through the sectoring phenomenon, occasional spores will yield more vigorous colonies; and the possibilities of this approach were here explored. Two strains showing the desired combinations of high penicillin yield and at least moderate sporulation were secured after selections had been carried out over three generations. Strains *53–414* and *53–399* have exhibited high productivity in shake-flask tests conducted in this laboratory. Average yields over 2500 O.U./ml have commonly been obtained (TABLE V) and titers have occasionally exceeded 3000 O.U./ml.

Unfortunately the most recent strains have not yet been extensively tested outside of the laboratory of their origin. According to present evaluations, however, the *51–20* side branch of the nitrogen mustard line constitutes the most superior stock thus far to emerge in the Wisconsin Family of *P. chrysogenum* strains.

DISCUSSION

As has already been intimated, the "Wisconsin Family" of *P. chrysogenum* strains by no means comprehends all the strains of penicillin-producing mold which have been developed and studied in this laboratory. According to the writers' definition, the "Family" includes only strain Wis. *Q176* and the select Wisconsin stocks derived therefrom. Excluded are all of the strains obtained in the early days of the Wisconsin project, as well as the thousands of variants secured in numerous experiments on mutation induction for which strain *NRRL 1951* has been used as a parental form.

In view of the fact that penicillin production has been one of the principal characters dealt with in the selection program out of which the Wisconsin Family of strains emerged, certain matters pertaining to the evaluation of strain potency must now be made clear. As is well known to those familiar with penicillin fermentation work, yields obtained with a given strain vary greatly under different fermentation conditions. The composition of the medium, the type of inoculum used, temperature, pH, the amount of aeration, and the type of precursor employed (if any) as well as the manner in which it is added are among the many factors which may profoundly influence the expression of a strain's basic capacity to produce the antibiotic. After extensive experimentation with fermentation conditions, Brown and Peterson (**7**) were able to secure yields in excess of 2000 O.U./ml from strain Wis. *Q176* —more than double the amount obtained during earlier tests in the same laboratory and three to four times as much as this strain usually produces in the laboratory of its origin. However, it is possible that even these high yields do not represent the maximum of which *Q176* is capable. Yields from a given strain may also vary appreciably from run to run, although every effort may have been made to keep fermentation conditions constant; and, in shake-flask tests, yields vary even among replicate flasks in a single run. Apparently such a minor detail as the relative tightness of a cotton plug may, by affecting aeration, influence the yield obtained. Variability among lots of corn steep liquor, used in the preparation of the medium, is believed to account for some of the yield dif-

ferences encountered over a period of time. It is also now well established that strains differ in their requirements for effective synthesis of the antibiotic and in their response to different fermentation conditions. Thus a given variant may perform in an outstanding fashion in one situation but appear to be mediocre in another, even though the latter may have been found favorable to some other strain. All of these considerations mean that evaluation of the abilities of a group of variants to produce penicillin is fraught with difficulties, that absolute potencies are rarely determined, and that ratings of cultures must to some extent at least be qualified in terms of fermentation conditions.

As has previously been explained, in the selection program in question here the evaluation of potency has been based upon a relatively uniform set of fermentation conditions—conditions, incidentally, which were probably far from optimum for most of the strains tested. As far as penicillin production has been concerned, then, this program has dealt with changes in potency as measured according to an arbitrary yardstick. In relation to the primary objectives of the study, that does not appear to have been a serious liability, but it is one which the reader should keep in mind.

Limited information on the performance of certain key strains of the Wisconsin Family in tests carried out elsewhere suggests that two interesting situations have developed simultaneously in connection with the emergence of this group of variants. It is evident, on the one hand, that the selection procedures followed have yielded a group of strains especially adapted to perform well under the fermentation conditions here employed, with the result that increases in potency registered by this laboratory's yardstick may not reflect a proportional change in basic potency or in ability to produce the antibiotic under other conditions (**29**). Thus, as has previously been noted, yields in the range of 2000–3000 O.U./ml have been secured in this laboratory with Wis. *51–20* and some other strains near the present end of the selection series, while the output of *Q176*, run as a control, has commonly been in the range of 400–650 O.U./ml—a situation which undoubtedly exaggerates the extent of the improvement in the newer strains. On the other hand, tests conducted outside of this laboratory have also indicated that in the Wisconsin Family of strains there have been realized some substantial gains in absolute potency, or at least increased productivity not confined to any narrowly restricted set of circumstances. There is far from perfect agreement in the rating of strains by this laboratory and by antibiotics workers elsewhere, but in the light of the situations discussed above this is hardly surprising. Perhaps the remarkable thing is that

the system of evaluation employed here has successfully picked out so many strains which later have come to be generally regarded as superior.

Although *P. chrysogenum* has now been studied intensively and by many individuals for over a decade, no way has yet been found to tell with certainty from the appearance of a culture what its capacities in regard to production of the antibiotic may be. Nevertheless, an extremely interesting and suggestive general correlation between cultural characteristics and potency in antibiotic production can be traced among the descendants of strain *NRRL 1951*. Beginning with strain *NRRL 1951·B25* and continuing through strains such as Wis. *50–935* and Wis. *51–20*, progressive increase in ability to produce the antibiotic, at least as measured by test conditions in this laboratory, has been paralleled by a more or less progressive reduction in vegetative and reproductive vigor. Strains rated highest are all extreme degenerates and weaklings compared with wild-type strains. Similar degenerative changes were reported by Farrell (**14**) in relation to her most productive strains. While it is unlikely that these relationships are entirely accidental or meaningless, it must be emphasized that it by no means follows that any degenerate strain will be found to be a good producer. Certainly among the survivors of mutagenic treatments one encounters many slow-growing and weakly-sporulating variants which have very poor capacity to synthesize the antibiotic. In this same connection it is important to bear in mind also the several instances in which substantial improvement in capacity to sporulate was secured in the Wis. *51–20* line without any loss of ability to produce penicillin.

The history of the Wisconsin Family of strains taken in conjunction with that of the ancestral stock would seem to indicate that, as far as the type of change which makes strains more attractive commercially is concerned, there is no one method of securing variants which is overwhelmingly superior. In this case each of the various techniques employed to obtain new strains has, in fact, made significant contributions to the building up of the stock. As will be recalled, the first improvements over the wild-type ancestor came with the selection of spontaneous variants by Raper and Alexander. The first significant improvements in the Wisconsin pigmentless stock were also obtained through spontaneous variation. Strains *47–638*, *47–1564*, and *48–701* represented progressive steps of improvement over strain *BL3–D10* and gave us the first industrially-attractive material in the pigmentless line. Later a spontaneous variant, *51–20*, initiated an attractive series as an offshoot from the nitrogen mustard branch of the family tree. To the credit of ultraviolet irradiation may be listed the following: the great improvement

represented by strain Wis. *Q176*, the initiation of the Wisconsin pigmentless series through production of race *BL3–D10*, and a substantial rise in potency in the latter part of the UV line of the three-way selection program. Interestingly enough, the one major contribution of the nitrogen mustard treatments came in the very first generation of the nitrogen mustard line. Unfortunately fewer generations have been followed through in this series than in the others. X-irradiation was not among the types of mutagenic treatments employed in securing variants in connection with the Wisconsin Family of strains; but strain *X–1612*, a product of X-irradiation, unquestionably embodies one of the outstanding improvements in the general stock to which this Family belongs. Possibly there is merit in integrating various types of mutagenic treatments and simple selection, as was somewhat inadvertently done in the case of the present *P. chrysogenum* stock. It will be recalled that Raper and Alexander (**41**), operating solely by selecting naturally-occurring variants, were unable to secure improvements beyond those represented in strain *NRRL 1951·B25*. However, after X-ray and UV treatments had established a new line of departure it was again possible to obtain advantageous changes by simple selection.

The outcome of the various UV irradiation treatments involved in the emergence of the Wisconsin Family of strains also fails to mark either of the two UV wave lengths employed or any particular dosage as especially advantageous. A wavelength of 2750Å was employed in the treatment which yielded *Q176*, while some of the rises in potency in the UV line of the three-way selection program were secured with radiation of 2534–37Å. As previously noted, studies on irradiation effects in strain *NRRL 1951* have indicated that the maximum number of morphological variants are obtained at a survival level of about 25%, and this general level gave satisfactory results in the three-way selection series. Yet it must be remembered that strain *Q176*, representing undoubtedly the greatest single step of improvement obtained through irradiation in this program and probably the greatest single step of progress in the development of the entire Wisconsin Family of strains, emerged following a severe treatment which left less than 1% of the spores able to form colonies. It should likewise be recalled that another highly important strain, *BL3–D10*, came into being followed an irradiation so gentle that no killing at all could be detected.

Finally it is to be emphasized that, even though a number of variants from the Wisconsin Family of strains have won important roles in the antibiotics industry, the selection program in which they emerged was designed primarily to serve the interests of pure science. It is believed

that much of fundamental interest has been learned by tracing the behavior of the organism through the long series of generations and treatments which have been involved. It seems likely that a knowledge of the evolution of this particular *P. chrysogenum* stock may be of value in appraising problems of variability in other imperfect fungi and provide perspective and tools for future workers desiring to obtain "tailor-made" strains of other organisms.

SUMMARY

A ten-year study on spontaneous and induced variation in penicillin-producing molds has yielded, in the writers' laboratories, an assemblage of superior variants which have become well known in industrial circles and which are referred to as the "Wisconsin Family" of *P. chrysogenum* strains. The origin, inter-relationships, and characteristics of key strains in this "Family" are here described.

The methods employed in obtaining, testing, and maintaining the improved stocks are considered. Strains embodying various advantageous changes have been secured through treatment of spores with ultraviolet radiation and with a nitrogen mustard. In addition, spontaneous changes have provided some improvements in the stock. Of the two wave lengths of ultraviolet employed in mutagenic treatments, neither has appeared to have any clear advantage over the other; and desirable changes were secured at high, moderate, and low UV dosage levels.

The Wisconsin Family begins with strain Wis. *Q176*. This strain, obtained in 1945, gave yields approximately double those obtainable from the best strain previously known. From this outstanding variant all other members of the Wisconsin Family trace their descent. The "Pigmentless Series," consisting of strains which secrete no yellow pigment, was inaugurated in 1947. In the "nitrogen mustard branch" of this series have emerged the best strains obtained in this selection program to date. Yields as high as 3000 O.U/ml have been secured from certain variants in this group under fermentation conditions employed in this laboratory. This is more than ten times the amount of penicillin produced under the same conditions by the improved strain from which the "Wisconsin Family" arose and about forty times the amount produced by the wild-type ancestor.

A more or less progressive decline in the vegetative and reproductive vigor of the strains has accompanied the increase in capacity to produce the antibiotic, not only in the Wisconsin Family itself but also in the succession of improved stocks which were ancestral to the Wisconsin

Family. Feeble sporulation and slow growth of the mycelium on agar are characteristic of all of the top-ranking strains. It is emphasized, however, that reduced vigor is not an infallible criterion for the selection of superior penicillin-producing variants. It is further emphasized that there is no close correlation between rate of colony growth on agar and rate of development of mycelium in aerated liquid cultures. In both small and large scale fermentations the best lines regularly produce their high yields about as quickly as maximum yields are obtained from wild-type or inferior strains.

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HYDROXYLATION OF STEROIDS, PRINCIPALLY PROGESTERONE, BY A STRAIN OF ASPERGILLUS OCHRACEUS

EUGENE L. DULANEY, EDWARD O. STAPLEY AND CHARLES HLAVAC

(WITH 5 FIGURES)

The ability of certain species of *Aspergillus* to transform progesterone to 11α-hydroxyprogesterone has been reported by the Upjohn (**7**) and Squibb groups (**4**) and has been observed in these laboratories. We find that the 11α-hydroxy derivative usually is transformed further to 6β,11α-dihydroxyprogesterone. Some cultures, however, were found to introduce secondary hydroxyls to 11α-hydroxyprogesterone at positions other than carbon 6. These findings as well as data characterizing the 11α-hydroxy and 6β,11α-dihydroxy derivatives of progesterone will be reported later (**2**).

In the present communication are reported our studies of progesterone transformation by a strain of *A. ochraceus* and particularly the effect of Zn^{++} on introduction of the 6β-hydroxyl to 11α-hydroxyprogesterone.

EXPERIMENTAL METHODS

The isolate of *A. ochraceus* used in this study was selected from a group of twenty isolates of several species that appeared to be promising on previous tests. These twenty isolates were grown in three media and tested quantitatively for transformation of progesterone to 11α-hydroxyprogesterone and 6β,11α-dihydroxyprogesterone.

The culture was grown for steroid conversion in 50 ml of medium in 250 ml Erlenmeyer flasks. Following autoclaving at 15 lbs pressure for 17 minutes, the medium was inoculated and flasks incubated at 28° C on rotary shakers moving at 220 rpm and describing a circle 1.5 inches in diameter. At the desired time the steroid was charged to each flask in 2.5 ml of propylene glycol. Unless otherwise noted the level of progesterone charged was always 10 mg per flask. For assay, the contents of a flask were homogenized for 1 minute in a Waring blendor and extracted three times with 25 ml amounts of chloroform. The extracts were dried, then taken up in pure methanol and aliquots spotted on

Whatman No. 1 filter paper. Chromatograms were developed using the solvent systems and methods of Zaffaroni and associates (**1**, **12**, **13**). The conversion products were located by means of an ultraviolet light scanner (**5**) and eluted from the paper strips in pure methanol. The optical densities of the eluates were determined at a wave length of 2400 Å and the amounts of the steroids present were calculated from these figures.

Further experimental details are given in the appropriate sections that follow.

RESULTS AND DISCUSSION

A typical progesterone conversion pattern using this strain of *A. ochraceus* is revealed in FIG. 1. The culture was grown in a medium of the following composition: blackstrap invert molasses, 50 g; corn steep liquor, 5 ml; and distilled H_2O to 1 liter. The pH was adjusted to 6.5 before autoclaving. The inference from this data is that progesterone is converted to 11α-hydroxyprogesterone and this derivative is further hydroxylated to form 6β,11α-dihydroxyprogesterone according to the scheme in FIG. 2.

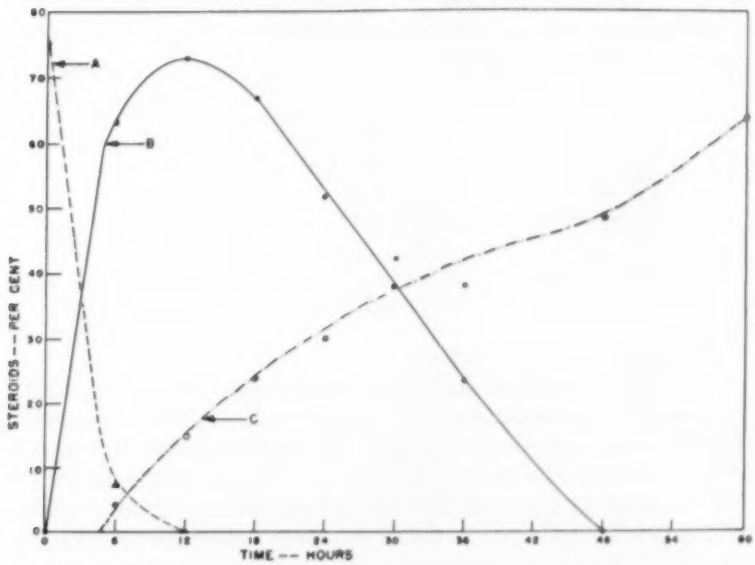

FIG. 1. Progesterone transformation by *A. ochraceus* in molasses-corn steep medium. Progesterone, curve A; 11α-hydroxyprogesterone, curve B; 6β,11α-dihydroxyprogesterone, curve C.

Assay of media in which *A. ochraceus* was grown but to which no sterol was added revealed no products that might be confused with these conversion products.

The desired conversion product of progesterone is 11α-hydroxyprogesterone; hence elimination of the 6β-hydroxylation of the 11α-hydroxy-

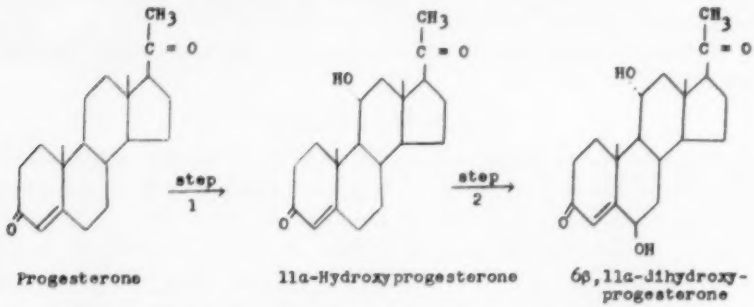

FIG. 2. Scheme for transformation of progesterone by *A. ochraceus*.

progesterone was desired. The results of a test of progesterone conversion by *A. ochraceus* grown in two synthetic media indicated that little 6β-hydroxylation occurred in one of these media. The compositions of these two media are as follows:

	No. 1	No. 2
Sucrose	5%	5%
NH_4NO_3	0.5%	
$NaNO_3$		0.76%
K_2HPO_4	0.65%	0.1%
$MgSO_4.7H_2O$	0.5%	0.05%
KCl		0.05%
$FeCl_3.6H_2O$	0.008%	
$FeSO_4.7H_2O$		0.001%
$ZnSO_4.7H_2O$	0.005%	
Distilled H_2O to	100%	100%

The results are shown in FIGS. 3 and 4.

The rapid conversion of the 11α-hydroxyprogesterone to 6β,11α-dihydroxyprogesterone in medium No. 1, FIG. 3, and the failure of the 6β-hydroxylation of the 11α-hydroxy derivative to occur to any great extent in medium No. 2, FIG. 4, stand out clearly. The only variable in this experiment is that of differences in medium composition; hence, the basis for the difference in the progesterone conversion patterns must be sought in the medium constituents.

The relationship of metals to enzyme systems prompted us to first test Zn^{++} and Fe^{++} as the factors responsible for effecting the 6β-hydroxy-

lation of 11α-hydroxyprogesterone. Synthetic medium No. 2 was supplemented with $ZnSO_4 \cdot 7H_2O$, 50 mg per liter, and $FeSO_4 \cdot 7H_2O$, 80 mg per liter, and tested as a medium for growth and progesterone transformation. The results, presented graphically in FIG. 5, show clearly that 11α-hydroxyprogesterone was further transformed to 6β,11α-dihydroxyprogesterone in this Zn^{++} and Fe^{++} supplemented medium. Experimental results detailed below show Zn^{++} to be the factor necessary for the 6β-hydroxylation.

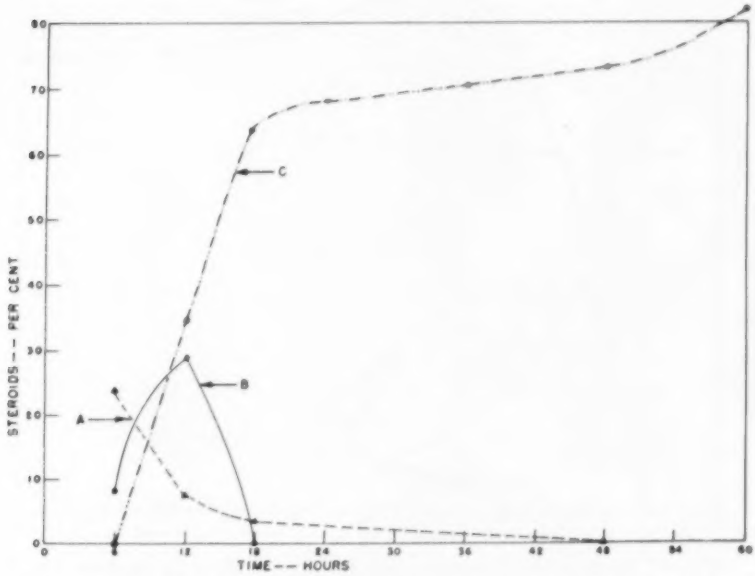

FIG. 3. Progesterone transformation by *A. ochraceus* in medium No. 1. Progresterone, curve A; 11α-hydroxyprogesterone, curve B; 6β,11α-dihydroxyprogesterone, curve C.

In studying the effect of Zn^{++} on 6β-hydroxylation of 11α-hydroxyprogesterone, considerable difficulty was encountered. *A. ochraceus* requires Zn^{++} for growth. This, coupled with the fact that a low level of Zn^{++} stimulates the 6β-hydroxylation, makes inoculum development quite critical. Production of the Zn^{++} deficient inoculum has been accomplished as follows. A good growth was obtained in synthetic medium No. 2 supplemented with 10 mgs per liter of $ZnSO_4 \cdot 7H_2O$. Growth from this flask was then carried through two or three passages in medium No. 2 unsupplemented with Zn^{++}. The growth in this last passage was

used as inoculum. At times three passages in the medium unsupplemented with Zn^{++} was not sufficient and use of this inoculum resulted in more 6β-hydroxylation than was normally obtained in Zn^{++} deficient medium. When this occurred, however, the 6β-hydroxylation started much later than in Zn^{++} supplemented medium and the level of the dihydroxy derivative obtained was always lower. Thus, the effect of Zn^{++} deficiency still could be observed.

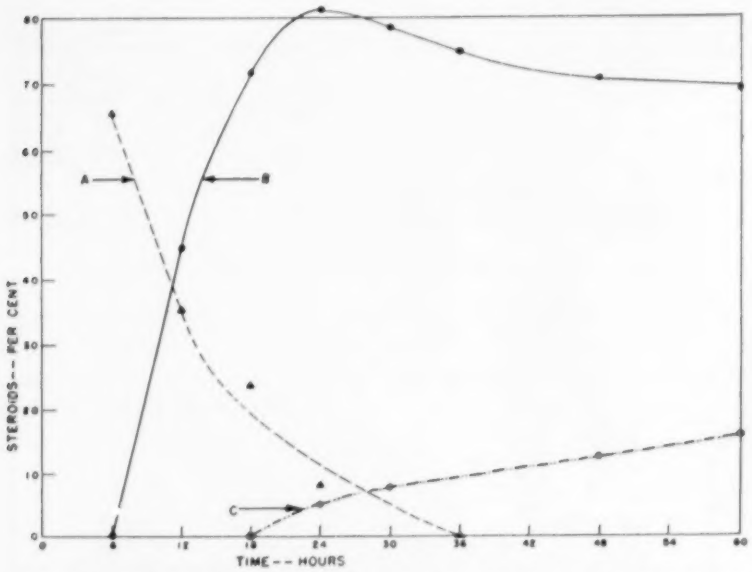

FIG. 4. Progesterone transformation by *A. ochraceus* in medium No. 2. Progesterone, curve A; 11α-hydroxyprogesterone, curve B; 6β,11α-dihydroxyprogesterone, curve C.

The specificity of Zn^{++} for the 6β-hydroxylation was demonstrated by testing a number of ions as single supplements to medium No. 2. Controls were run with Zn^{++} supplemented and non-supplemented medium. The inoculum used was Zn^{++} deficient. The compounds tested as single supplements were as follows: aluminum sulfate, antimony chloride, barium sulfate, bismuth chloride, sodium borate, calcium chloride, lead acetate, lithium hydroxide, ammonium molybdate, nickel sulfate, silicic acid, silver sulfate, stannous chloride, strontium chloride, sodium tungstate, and zinc sulfate each at a level of 40 mgs per liter. Also tested were cadmium sulfate, copper sulfate, cobalt nitrate and chromic acid

each at 20 mgs per liter, and mercuric sulfate at 10 mgs per liter. These concentrations exceeded solubilities of the salts in some instances. Silver sulfate and cadmium sulfate were toxic at these concentrations. The results are too extensive to report in detail but may be summarized as follows: All treatments to which the various ions were added, with the

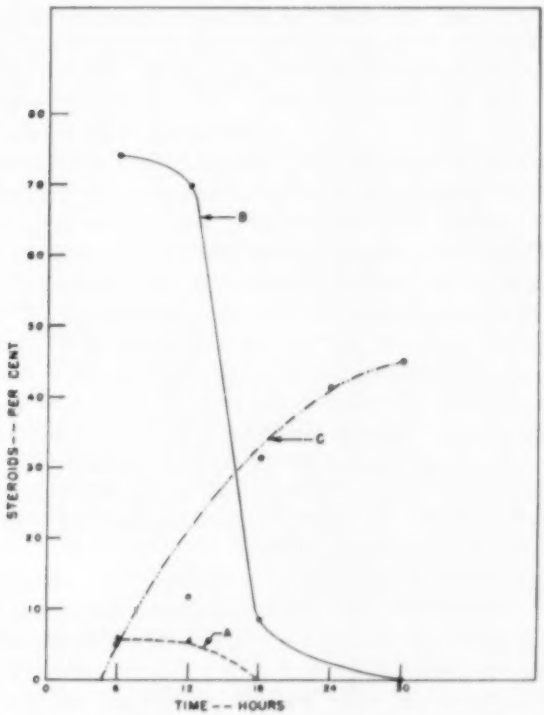

FIG. 5. Progesterone transformation by *A. ochraceus* grown in medium No. 2 supplemented with Zn^{++} and Fe^{++}. Progesterone, curve A; 11α-hydroxyprogesterone, curve B; 6β,11α-dihydroxyprogesterone, curve C.

exception of Zn^{++}, resulted in conversions that agreed well with the conversion obtained in the unsupplemented control medium, i.e., there was little 6β-hydroxylation of the 11α-hydroxyprogesterone.

$CdSO_4 \cdot 8H_2O$ was toxic at a level of 20 mgs per liter. The effect of this salt on growth and progesterone conversion was then determined at levels of 1 mg, 5 mgs, 10 mgs, and 15 mgs per liter. In this experiment a Zn^{++} deficient inoculum was not used and an unexpected effect, perhaps

antagonism between Zn^{++} and Cd^{++}, was found at the higher levels of Cd^{++}. At levels of 1 mg per liter and 5 mgs per liter, there was no demonstrable effect of Cd^{++} on the 6β-hydroxylation of 11α-hydroxyprogesterone. At levels of 10 mgs per liter and 15 mgs per liter, particularly at the 15 mgs per liter level, the $CdSO_4 \cdot 8H_2O$ in the presence of Zn^{++} markedly inhibited the 6β-hydroxylation. This may be due to antagonism between the Zn^{++} and Cd^{++} ions, but another explanation should be considered. At a level of 20 mgs per liter $CdSO_4 \cdot 8H_2O$ is toxic. Growth is somewhat inhibited at a level of 15 mgs per liter, but not markedly so. The inhibition of 6β-hydroxylation by Cd^{++} at these higher levels could be due to its toxic effect on protein synthesis, thus limiting the formation of the adaptive enzyme necessary for 6β-hydroxylation. The slower rate of progesterone utilization in the treatments containing 10 mgs per liter and 15 mgs per liter of $CdSO_4 \cdot 8H_2O$ would seem to support this hypothesis. Conversely, however, growth did occur at these levels of Cd^{++} and high levels of 11α-hydroxyprogesterone were produced. The 11α-hydroxylation is carried out also by an adaptive enzyme.

A priori, the effect of Zn^{++} on the conversion of 11α-hydroxyprogesterone to the 6β,11α-dihydroxy derivative could be due to one of several possible mechonisms. Zn^{++} may be a co-factor of the enzyme necessary for the 6β-hydroxylation. Another possibility is that in Zn^{++} deficient cells, an inhibitor is formed that suppresses the 6β-hydroxylation. A third possibility is that we are using a well balanced system in which just enough Zn^{++} is added to allow a definite, but limited amount of protein synthesis and hence adaptive enzyme formation.

The replacement method was used to study the mechanism of the Zn^{++} effect. The culture was grown in medium No. 2 and adapted to progesterone for 6 hours before use. After adaptation, the medium was decanted and the cells washed well with a sodium hydroxide-potassium biphthalate buffer at pH 4.0. The cells were resuspended in 50 ml of fresh buffer and the progesterone was added. No other factors need to be added in this replacement save buffer and the steroid to be transformed. Nicotinamide was found not to be stimulatory.

Using the replacement method it was found that in order for 6β-hydroxylation of 11α-hydroxyprogresterone to proceed the organism must be grown in the presence of Zn^{++}. Growing Zn^{++} deficient cells and adding Zn^{++} to the replacement had no effect on the formation of the 11α-hydroxyprogesterone but did not allow further conversion to the 6β,11α-dihydroxy compound.

The possibility that Zn^{++} was required specifically as a co-factor for 6β-hydroxylation was investigated by studying conversion of 11α-hy-

droxyprogesterone. *A. ochraceus* was grown in medium No. 2 containing Zn^{++}, and in medium deficient for Zn^{++}, then adapted to 11α-hydroxyprogesterone. By use of the replacement method these Zn^{++} deficient and Zn^{++} sufficient cells, adapted to 11α-hydroxyprogesterone, were used to study 6β-hydroxylation of 11α-hydroxyprogesterone. The only difference that could be noted was that the Zn^{++} deficient cells formed the 6β,11α-dihydroxy compound somewhat slower in the early stages of the conversion than did the cells grown in medium containing Zn^{++}. After 48 hours, however, approximately the same level of the dihydroxy derivative was obtained with both sets of cells. This would indicate that Zn^{++} is not a co-factor in the 6β-hydroxylation.

The possibility that an inhibitor of 6β-hydroxylation was formed in Zn^{++} deficient cells could not be confirmed experimentally. *A. ochraceus* was grown in medium No. 2 supplemented with $ZnSO_4 \cdot 7H_2O$, adapted to progesterone for 6 hours, washed with buffer, then suspended in fresh buffer plus 10 mg of progesterone per flask. Ten ml of a homogenate of Zn^{++} deficient cells was added to some of the flasks and no homogenate to others. Special care was taken to insure that the homogenized growth was Zn^{++} deficient by carrying it through five passages in Zn^{++} deficient medium. At the time the cells were washed and homogenized in buffer, they were becoming necrotic. The results of this experiment certainly indicated no inhibition of the 6β-hydroxylation by the Zn^{++} deficient homogenate. If anything, conversion of the 11α-hydroxy derivative to the dihydroxyprogesterone was more rapid in the flasks containing the homogenate. Our experiments, however, do not exclude the possibility of a very unstable inhibitor being present in Zn^{++} deficient cells. It might be argued that growth of cells in a Zn^{++} deficient medium stimulates the formation of some enzyme that destroys a co-factor of the 6β-hydroxylating system. For example, Nason *et al.* (**8**, **9**) found Zn^{++} deficient cells of *Neurospora* to be rich in DPNase and to show decreased levels of alcoholic dehydrogenase and a reduced synthesis of tryptophan. This certainly shows that Zn^{++} and perhaps other metal deficiencies induce marked abnormalities in enzyme systems. A situation analogous to this might be operating in Zn^{++} deficient cells of *A. ochraceus*. However, the results with Zn^{++} deficient homogenates coupled with the ability of Zn^{++} deficient cells, when adapted to 11α-hydroprogesterone to convert this to the 11α,6β-dihydroxy derivative, would argue against this possibility.

Our results suggest that the failure of Zn^{++} deficient cells to 6β-hydroxylate the 11α-hydroxyprogesterone that they have formed from progesterone is due to a lack of formation of the required adaptive enzyme. Furthermore, the failure to form this enzyme is not due to the

TABLE I

CONVERSION OF VARIOUS LEVELS OF PROGESTERONE BY ASPERGILLUS OCHRACEUS IN SYNTHETIC MEDIUM NO. 2

Progesterone added, mg/50 ml medium per flask	Age of sample, hrs	Progesterone, % recovered	% Conversion to 11α-hydroxy-progesterone	% Conversion to 6β, 11α-dihydroxy-progesterone
10	6	21.5	28.8	0
	12	9.4	87.2	0
	18	0	70	5.6
	24	0	85.5	15
	46	0	54.1	13.1
20	6	28.5	22.6	0
	12	28.4	55.3	0
	18	11.6	65.8	0
	24	0	74.6	8.5
	46	0	62.7	6.8
	70	0	62.3	11.7
30	6	57.9	8.5	0
	12	32.6	29.5	0
	18	26.6	44.3	0
	24	11.3	66.4	3
	46	4.0	70.9	8.8
	70	0	64.5	8.9
40	6	61.7	2.8	0
	12	55.5	17.9	0
	18	18.4	47.8	0
	24	32	42	0
	46	0	79.9	6.8
	70	0	66.7	4.3
50	6	56	2.4	0
	12	37	16.5	0
	18	33.2	32	0
	24	32.4	39.7	0
	46	12.3	56	2.6
	70	12.3	72.1	4.0
75	6	56.5	3.2	0
	12	34.1	9.2	0
	18	45.5	23.6	0
	24	41.7	21.4	0
	46	38.7	49.1	0
	70	15	61.5	1.6
100	6	53.5	0	0
	12	57.2	7.2	0
	18	61.7	10.8	0
	24	58.5	16.1	0
	46	30.5	41.3	0
	70	25.8	39.4	0

fact that Zn^{++} is a co-factor of the enzyme or that an inhibitor in Zn^{++} deficient cells is inactivating the enzyme when it is formed. The probable explanation is that we are using a well balanced system in which enough Zn^{++} is added to allow the organism to grow and thus bind most of the

Zn^{++}. When progesterone is added to the system, apparently just enough free Zn^{++} is available to allow a limited amount of protein synthesis and the adaptive enzyme necessary for 11α-hydroxylation of the progesterone is formed. There is not enough free Zn^{++} to allow further protein synthesis and the second adaptive enzyme necessary for 6β-hydroxylation is not formed. It is also possible that nitrogen and carbon would be limiting protein synthesis, too, for in buffer replacement systems the source of nitrogen and carbon must be endogenous. The exact site or sites of action of the Zn^{++} are not known; however, three possible sites of Zn^{++} action may be noted (**3, 10, 11**).

In all of the above experiments, the progesterone to be converted was added at a level of 10 mgs per 50 mls of medium or buffer per flask. An experiment was set up to determine just how much progesterone could be converted by the amount of cells grown in 50 mls per flask of synthetic medium No. 2 unsupplemented with Zn^{++}. The culture was grown 4 days prior to sterol addition. The buffer replacement method was not used. The results are summarized in TABLE I.

The data in TABLE I clearly indicate that levels of progesterone higher than 10 mgs per 50 mls of medium can be converted. For example, 72.1% (or 35 mgs) of the 50 mgs added to 50 mls of medium was converted to 11α-hydroxyprogesterone after 70 hours and 61.5% (or 46 mgs) of the 75 mgs added to 50 mls of medium was converted after 70 hours. An interesting observation may be made from these data. The amount of 6β,11α-dihydroxyprogesterone formed and the time of its appearance are affected by the level of progesterone. The dihydroxy derivative appears later and the level obtained is decreased when the amount of unreacted progesterone remains high.

Other Steroids. In addition to progesterone, *A. ochraceus* is capable of transforming other compounds. Substance S (11-desoxy-17-hydroxycorticosterone) is converted to epi F in yields of approximately 50%, and 11-desoxycorticosterone (DOC) is converted to epi-corticosterone.

TABLE II

CONVERSION OF DOC (10 MG/FLASK) BY ASPERGILLUS OCHRACEUS IN THE BUFFER REPLACEMENT SYSTEM

Age of sample, hrs	DOC % recovered	% Conversion to epicorticosterone
1	76.9	11.1
2	48.5	15.3
4	59.7	11.6
8	18.4	67.6
16	4.0	78.7
32	3.5	85.3
46	6.1	75.7

Conversion of DOC to epi-corticosterone in a buffer replacement experiment is summarized in TABLE II.

SUMMARY

A strain of *Aspergillus ochraceus* has been studied in detail for conversion of progesterone. This strain converts progesterone to 11α-hydroxyprogesterone and this derivative is further hydroxylated to 6β, 11α-dihydroxyprogesterone. The 6β-hydroxylation of 11α-hydroxyprogesterone can be prevented by a Zn^{++} deficiency. This has been explained by the limitation placed on protein synthesis and adaptive enzyme formation by a deficiency of Zn^{++}.

Conversion of other steroids by *A. ochraceus* is noted.

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THE NUTRITION OF TRICHOPHYTON TONSURANS

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(WITH 5 FIGURES)

Trichophyton tonsurans is an important agent of epidemic ringworm in many areas of the world, causing a disease which may involve the hair, skin, and nails. Infections occur in both children and adults and these are often extremely difficult to cure. During the past eight years, ringworm due to *T. tonsurans* has become a health problem in the United States, and better methods of diagnosis, treatment, and control are needed (**7**).

Accurate diagnosis of *T. tonsurans* ringworm cannot be made on a clinical basis alone, but is dependent upon microscopic examination of clinical materials, coupled with the isolation and identification of the causative agent. The final diagnosis rests, therefore, on laboratory studies of the fungus itself. The usual procedure is to identify the fungus on the basis of its gross and microscopic morphology as observed on a standard medium such as Sabouraud dextrose agar. Experience in this laboratory and others indicates, however, that this is often difficult. This is because *T. tonsurans* grossly presents a highly varied morphology, and microscopically reveals no distinctive spore form or spore arrangement which can be completely relied upon for identification purposes. Identification must depend, therefore, upon a group of rather variable characteristics. Furthermore, certain strains may be difficult to identify because other dermatophyte species, particularly *Trichophyton mentagrophytes* and *Trichophyton rubrum*, may at times present similar morphological characteristics.

The present investigation was undertaken in order to learn the fundamental nutritional requirements of *T. tonsurans*. It was hoped that nutritional studies of *T. tonsurans* would reveal certain physiological characteristics of the organism which would be of value in the laboratory identification of this fungus. Such studies perhaps would supply also some information concerning the general physiology of this fungus and of its ability to invade tissues.

The nutrition of *T. tonsurans* has been studied previously. In 1943,

Burkholder and Moyer (**1**) found one strain of *T. acuminatum* (*T. tonsurans*) to be "partially deficient for thiamine." They also reported one strain, *T. sulfureum* (*T. tonsurans*), to be deficient for thiamine. Drouhet and Mariat (**4**) reported a total deficiency for thiamine in five strains of the *tonsurans* group (this included the following varieties: *acuminatum, crateriforme,* and *sulfureum*). Drouhet (**2**) reported little or no growth of these strains on inorganic nitrogen. Sullivan, Bereston and Wood (**15**), working with fifteen isolates of *T. tonsurans,* found a partial thiamine deficiency. They, however, reported fair growth on inorganic nitrogen, and Wood (**16**) found one isolate to be deficient for ornithine, citrulline, or arginine.

MATERIALS AND METHODS

Fifty strains of *T. tonsurans* were used in this investigation. Complete studies were made using sixteen apparently typical, recently isolated strains, and two strains each of *T. mentagrophytes* and *T. rubrum* which served as controls. On the basis of the findings in this test group, a survey was made of thirty-four other strains of *T. tonsurans* obtained from various culture collections. Many of these later strains are listed under a wide variety of names but are believed on the basis of morphological studies by one of us (**9**) to be synonyms of *T. tonsurans.* A list of all these strains with their sources is presented in TABLE VII.

The basal medium used in these studies was made up as follows:

10% acid hydrolyzed, vitamin-free casein	50.0 ml
Dextrose C. P.	40.0 gms
Purified agar	15.0 gms
$MgSO_4$ C. P.	0.1 gm
Sorensen's phosphate buffer solution at pH 6.8	200.0 ml
Trace elements solution[1]	0.5 ml
Distilled water (q.s.)	1000.0 ml

Different carbon and nitrogen compounds were substituted for dextrose and casein as noted in the various experiments.

The medium was standardized to pH 6.8 before autoclaving at 120° C for ten minutes. After autoclaving, the pH of the medium was approximately 6.5.

When needed, vitamins were added aseptically as Seitz-filtered solutions to the partially cooled medium after autoclaving. The final concentration of vitamins per ml of medium was as follows:

[1] Trace element solution: H_3BO_3, 28.5 mg; $CuSO_4 \cdot 5H_2O$, 93.0 mg; MoO_3 (85%), 17.6 mg; $Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$, 865.0 mg; $MnSO_4 \cdot H_2O$, 30.5 mg; $ZnSO_4 \cdot 7H_2O$, 395.0 mg; distilled water (q.s.), 500.0 ml.

Biotin (free acid) ..	0.002 microgram	i-Inositol	500.0 micrograms
Thiamine HCl	0.2 microgram	Nicotinic acid	1.0 microgram
Riboflavin	1.0 microgram	Nicotinamide	1.0 microgram
Ascorbic acid	0.2 microgram	p-Aminobenzoic acid	5.0 micrograms
Choline Cl	5.0 micrograms	Ca. pantothenate ...	0.2 microgram
Folic acid	5.0 micrograms	Pyridoxine HCl ...	0.2 microgram

The media were poured into tubes in 5 ml amounts and slanted. They were examined 14 and 21 days after inoculation. Growth was recorded on the basis of colony diameter measurements or, when quantitative measurements were not necessary, growth was estimated and recorded as 1 +, 2 +, 3 +, or 4 + as compared with controls or other members of a series.

In the vitamin experiments, acid-clean glassware was used entirely and the agar was purified by washing it in distilled water fifteen times and then extracting it with hot 95% alcohol.

The purity of the vitamin-deficient media was checked with appropriate microorganisms when available. These were *Phycomyces blakesleeanus* for thiamine, *Pythiomorpha gonapodyoides* for pyrimidine, *Mucor ramannianus* for thiazole, *Neurospora crassa* for biotin, and *Saccharomyces cerevisiae* for inositol.

Auxanograms, according to the method described by Lodder (**11**), were used in order to demonstrate thiamine deficiency by use of the thiamine antagonist, neopyrithiamine.

EXPERIMENTAL DATA

Hydrogen ion and temperature tolerance. In order to conduct the experiments under optimum conditions, pH and temperature ranges of the fungus were studied. This was done by using buffered and unbuffered solid media at different pH values. On routine Sabouraud dextrose agar (Difco, unbuffered), when the initial pH ranged from 4.5 to

TABLE I

GROWTH OF TRICHOPHYTON TONSURANS AS A FUNCTION OF pH

Buffered Sabouraud dextrose-agar		Unbuffered Sabouraud dextrose-agar		Unbuffered ammonium nitrate agar	
pH	14 days	pH	14 days	pH	28 days
4.2	0	4.5	3–4+	4.1	Trace
4.8	2+	4.8	4+	4.8	1+
5.9	4+	5.4	4+	6.0	2+
7.1	2+	7.2	4+	6.7	1+
8.0	Trace	8.7	4+	7.3	Trace

8.7, good growth occurred in all tubes, as shown in TABLE I. On buffered Sabouraud dextrose agar, however, good growth occurred only over a pH range of 4.8 to 7.1 with a sharp maximum occurring at about pH 6.0. Similar results were obtained by Peck and Rosenfeld with several dermatophyte species (**12**).

With the inorganic nitrogen source, ammonium nitrate, a similar growth response to pH was obtained. Using unbuffered ammonium nitrate with dextrose agar, good growth occurred only at pH 6.0 (FIG. 1). The desired pH values of the buffered media, as determined by a glass electrode pH meter, were obtained by varying the proportion of M/15 KH_2PO_4 and Na_2HPO_4 buffer solutions.

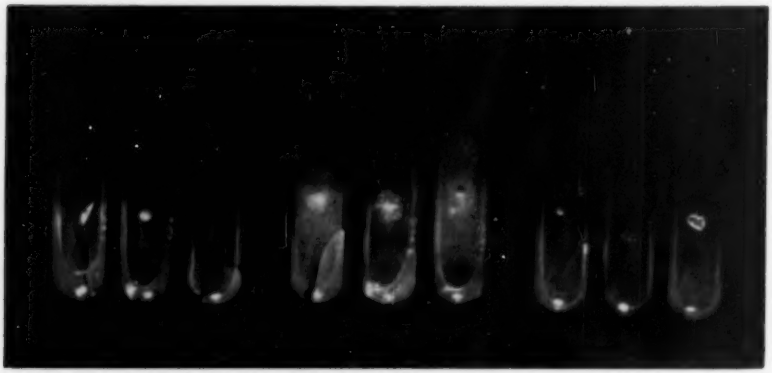

FIG. 1. Growth of *T. tonsurans* strains No. 1, 3, and 4 at different pH values on unbuffered ammonium nitrate agar. Left to right, pH 4.1, 6.0, and 7.3.

In the experiments with unbuffered media the pH was adjusted by adding sterile NaOH or HCl after autoclaving.

The effects of temperature on growth were determined by growing *T. tonsurans* on Sabouraud dextrose agar slants at 25°, 30°, and 37° C. At the end of 14 days the greatest amount of growth, as measured by colony size and density, had occurred at 30° C. In subsequent experiments all cultures were incubated at this temperature. Strain number 2, which showed a yellow pigment at 25° and 30° C, developed a red pigment at 37° C.

Nitrogen requirements. In order to develop the best basal medium for further work, nitrogen utilization studies were carried out. The test organisms were grown on the following media:

(1) Acid-hydrolyzed, vitamin-free casein and a mixture of the twelve water-soluble vitamins.

(2) Acid-hydrolyzed, vitamin-free casein minus the vitamin mixture.
(3) Ammonium nitrate plus the vitamin mixture.
(4) Ammonium nitrate minus the vitamin mixture.
(5) Ammonium chloride plus the vitamin mixture.
(6) Potassium nitrite plus the vitamin mixture.
(7) Urea plus the vitamin mixture.

The nitrogen source was added at the rate of 0.5 gm per liter. As shown in TABLE II, the vitamin-supplemented casein supported the best growth. The ammonium nitrate medium without vitamins supported very poor growth. The addition of the vitamin mixture to this medium increased the growth of all strains. Ammonium chloride plus vitamins, and urea plus vitamins, gave similar results when compared with the ammonium nitrate plus vitamin medium. Potassium nitrite supported no growth.

TABLE II

COLONY DIAMETER OF TRICHOPHYTON TONSURANS STRAIN NO. 5 ON DIFFERENT NITROGEN SOURCES

Nitrogen source	Diameter in mm. 25 days
Casein + vitamins	34.0 mm.
Casein − vitamins	9.0 mm.
NH_4NO_3 + vitamins	11.0 mm.*
NH_4NO_3 − vitamins	4.0 mm.*
NH_4Cl + vitamins	10.0 mm.*
Urea + vitamins	12.0 mm.
KNO + vitamins	0.0 mm.

* Growth on inorganic nitrogen was more thin and submerged than on an organic nitrogen source.

Later experiments, using ammonium sulfate and sodium nitrate as a nitrogen source, indicated that the ammonium ion is that portion of the ammonium nitrate molecule that supports the best growth. Practically no growth occurred on the medium with sodium nitrate as a nitrogen source, while good growth occurred with ammonium sulfate as the nitrogen source. All strains showed the same response.

Vitamin requirements. On the basis of the nitrogen assimilation tests, acid-hydrolyzed casein was used as the nitrogen source for the experiments with water-soluble vitamins. Stock solutions of vitamins were prepared so that a different vitamin was absent from each of eleven mixtures. Biotin was prepared and added separately. These vitamin solutions were Seitz-filtered and were added aseptically to the tubed, partially cooled, vitamin-free casein medium. Therefore, all vitamins were tested by omission from an otherwise complete mixture. These tests were confirmed by single vitamin additions to the vitamin-free casein

medium. The first inoculum was taken from the surface of Sabouraud dextrose agar slants; all experiments were repeated at least once, and subsequent inoculations were made from the preceding series, which was free of the vitamin being tested. There was little difference in the colony size of the first and second transfer, indicating that no significant amounts of vitamin had been transferred with the inoculum.

These studies indicated that only thiamine stimulated the growth of *T. tonsurans*, as demonstrated in TABLE III. FIG. 2 shows the stimulation of growth on both NH_4NO_3 and casein media by the addition

TABLE III

COLONY DIAMETER OF A NORMAL AND A PLEOMORPHIC STRAIN OF TRICHOPHYTON TONSURANS ON DIFFERENT VITAMIN SUBSTRATES AFTER 21 DAYS

Substrate	Normal Strain No. 7	Pleomorphic Strain No. 15*
Plain casein	6 mm	18 mm
Casein plus all vitamins	35 mm	48 mm
Casein plus all vitamins except biotin	30 mm	48 mm
Casein plus all vitamins except thiamine	10 mm	24 mm
Casein plus all vitamins except riboflavin	37 mm	45 mm
Casein plus all vitamins except ascorbic acid	42 mm	50 mm
Casein plus all vitamins except choline	37 mm	45 mm
Casein plus all vitamins except folic acid	36 mm	45 mm
Casein plus all vitamins except inositol	37 mm	45 mm
Casein plus all vitamins except nicotinic acid and nicotinamide	35 mm	47 mm
Casein plus all vitamins except p-amino-benzoic acid	34 mm	47 mm
Casein plus all vitamins except calcium pantothenate	40 mm	48 mm
Casein plus all vitamins except pyridoxine	38 mm	47 mm

* Strain No. 15 is a pleomorphic variant of Strain No. 7.

of thiamine. It was also apparent that the pleomorphic (nonsporulating) strains were much less deficient for thiamine; that is, they were more autotrophic and consistently made better growth on all vitamin mixtures. FIG. 3 shows a comparison of the amounts of growth produced by normal and pleomorphic strains of *T. tonsurans* on thiamine-free media.

In order to determine the degree of thiamine deficiency, a thiamine titration study was done. In this experiment a liquid vitamin-free casein medium was made by omitting the agar from the casein medium described above. This liquid medium was tubed in 5 ml amounts and sterilized by autoclaving. A series of 20 tubes was used. To the first tube was added 5 micrograms of thiamine (in one-half ml of solution) and enough liquid to make 10 milliliters. Five ml from this first tube were then

FIG. 2. Growth of *T. tonsurans* on NH_4NO_3 and casein agar with and without added thiamine. Left to right, (1) NH_4NO_3, (2) NH_4NO_3 + thiamine, (3) casein, (4) casein + thiamine.

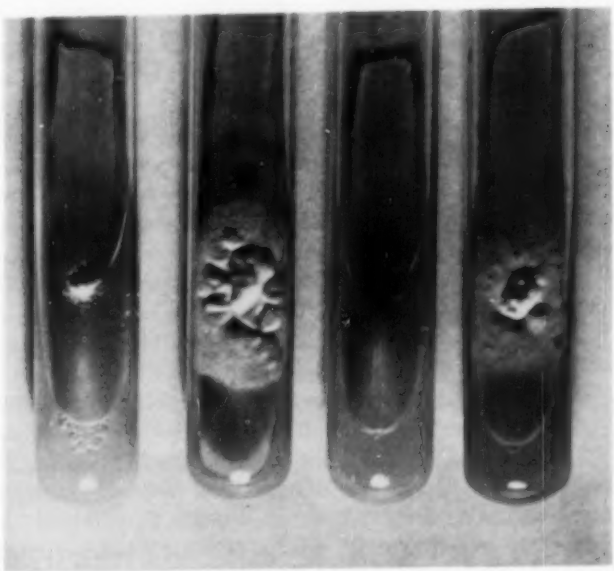

FIG. 3. Two normal strains of *T. tonsurans* (left to right 1 and 3), and two pleomorphic strains derived from them (left to right 2 and 4) growing on thiamine-free media.

transferred to the second tube, and the dilution series continued by successive halving. This procedure resulted in 2.5 micrograms of thiamine per 5 ml in the first tube, 1.25 micrograms in the second, 0.62 in the third, etc. These tubes were then inoculated by loop with a spore suspension from *T. tonsurans* strain No. 6. Growth after 14 days was visually estimated as trace, 1 + to 4 + when compared with minus thiamine and plus thiamine controls.

This thiamine titration indicated that even 0.000003 microgram per 5 ml produced slightly more growth than the thiamine-free controls. The lowest concentration of thiamine required to produce maximum growth was approximately 0.02 microgram per 5 milliliters.

Auxanogram studies with neopyrithiamine, a synthetic thiamine antagonist, showed that it inhibited *T. tonsurans* and *P. blakesleeanus* at about the same concentration. As *P. blakesleeanus* is a known thiamine-deficient organism, this confirms the results of the above studies that *T. tonsurans* is thiamine-deficient.

At this time attempts were made to determine whether the thiamine effect was a result of a total or partial deficiency. Various methods of further purifying the medium were tried and it was found that by adding 5 grams of activated charcoal per liter and filtering after autoclaving, a subtrate was obtained that permitted considerably less growth than the untreated vitamin-free casein (**10**). Repeated transfers on this purified medium, however, still showed some growth, although the amounts varied with the different strains. Some strains produced submerged growth extending less than 2 mm from the point of inoculation on the thiamine-deficient medium, while the pleomorphic varieties developed almost normally. It was not possible, however, to show a complete thiamine deficiency for any strain of *T. tonsurans*.

The two components of thiamine, pyrimidine and thiazole, were tested to determine what part or parts of the thiamine molecule were utilized for growth. It was found (Table IV) that 2-methyl-4-amino-5-aminoethyl pyrimidine would substitute for thiamine as would 2-methyl-5-ethoxymethyl-6-amino pyrimidine; 2-amino-4-methyl pyrimidine would not substitute for thiamine, nor would 2,6-dihydroxy-4,5-diamino pyrimidine or 2,4-diamino-6-hydroxy pyrimidine. Thiazole as 4-methyl-5-betahydroxyethyl thiazole would not substitute for thiamine when used alone. When used with one of the active pyrimidines, however, good growth occurred when tested with *T. tonsurans* and the controls *P. blakesleeanus* and *P. gonapodyoides*. The pyrimidine and thiazole compounds were used at a concentration of 1 microgram per 5 milliliters.

The four control cultures, two strains of *T. mentagrophytes* and two strains of *T. rubrum*, were tested along with the *T. tonsurans* strains for vitamin deficiencies. Neither of the *T. mentagrophytes* or *T. rubrum* strains showed any deficiency.

Amino acid utilization studies. Amino acid utilization was studied by growing the test organism on a complete amino acid medium and then comparing with growth obtained after omitting various groups of amino acids. The amino acids tested were: glycine, DL alanine, beta alanine, DL serine, DL threonine, DL valine, L leucine, DL isoleucine, DL norleucine, L proline, L tryptophane, L tyrosine, DL phenylalanine,

TABLE IV

GROWTH-PROMOTING EFFECT OF PYRIMIDINE AND THIAZOLE COMPOUNDS

Compounds*	*T. tonsurans*	*P. gonapodyoides***	*P. blakesleeanus****
2-methyl-4-amino-5-amino ethyl pyrimidine	4+	4+	0
2-methyl-5-ethoxymethyl-6-amino pyrimidine	4+	4+	0
2,6-dihydroxy-4,5-diamino-pyrimidine	0	0	0
2,4-diamino-6-hydroxy-pyrimidine	0	0	0
2-amino-4-methyl-pyrimidine	0	0	0
4-methyl-5-betahydroxy-ethyl thiazole	0	0	0
4-methyl-5-betahydroxy-ethyl thiazole + one active pyrimidine	4+	4+	4+

* Test compounds used in a concentration of 1 microgram/5 ml.
** *P. gonapodyoides* requires pyrimidine.
*** *P. blakesleeanus* requires thiazole + pyrimidine.

L lysine, L arginine, L histidine, DL ornithine, L cystine, L cysteine, DL methionine, L aspartic acid, L glutamic acid, L asparagine, L glutamine, and L hydroxyproline. The amino acid L hydroxyproline was omitted from Group I, which contained all other aminoacids, because L hydroxyproline has been reported as being toxic to dematophytes (**13**). Successive groups of amino acids were then omitted from groups II, III, IV, V, and VI. The components of the various amino acid test groups are listed in TABLE V. The amino acids in group I were added so that 50 mg of nitrogen were present in 100 ml of medium. In the following groups, as different amino acids were omitted, the concentrations of the remaining amino acids were increased so that the total nitrogen concentration always remained the same. The amino acids were added to the partially cooled basal agar as buffered aqueous solutions that had been sterilized by autoclaving (or by filtration if glutamine was present). In these experiments thiamine was added at the rate of 0.2 microgram

per ml, so that this factor was not limiting. As shown in TABLE V the mixture containing all the amino acids supported the best growth.

Most of the amino acids were studied singly by measurement of colony diameters of 5 typical strains of *T. tonsurans* on a medium containing only one amino acid. This amino acid medium (one for each different amino acid) was prepared by adding the amino acid to the basal agar as the sole nitrogen source in a concentration sufficient to provide 0.2 grams of nitrogen per liter. The DL amino acids were added in double amounts (i.e., in amounts sufficient to provide 0.2 grams of nitrogen by the L form). As indicated in TABLE VI, L arginine and

TABLE V

GROWTH OF TRICHOPHYTON TONSURANS ON DIFFERENT AMINO ACID MIXTURES

Amino acid media	Strain No. 5* Colony diameter after 21 days
Group I—All amino acids except hydroxyproline	30 mm
Group II—Contains methionine, cysteine, cystine, ornithine, histidine, arginine, lysine, phenylalanine, tryosine, tryptophane, proline, norleucine, isoleucine, valine, threonine, serine, beta alanine, alanine, glycine	17 mm
Group III—Contains ornithine, histidine, arginine, lysine, phenylalanine, tyrosine, tryptophane, proline, norleucine, isoleucine, leucine, valine, threonine, serine, beta alanine, alanine, and glycine	13 mm
Group IV—Contains phenylalanine, tyrosine, tryptophane, proline, norleucine, isoleucine, leucine, valine, threonine, serine, beta alanine, alanine, and glycine	10 mm
Group V—Contains norleucine, isoleucine, leucine, valine, threonine, serine, beta alanine, alanine, and glycine	13 mm
Group VI—Contains threonine, serine, beta alanine, alanine, and glycine	8 mm

* Data given for one strain, as there was no significant difference of other normal strains. Strain number 2 made no growth on amino acid mixtures lacking arginine and ornithine.

L ornithine supported the best growth. The amino acids L proline, DL serine, DL, alanine, L cystine, and DL phenylalanine supported good growth. The amino acids glycine, L tyrosine, L leucine, L tryptophane, and DL valine supported fair growth, while L hydroxyproline, L lysine, DL isoleucine, L histidine, DL methionine, DL threonine, and DL norleucine supported little or no growth.

The amino acid L hydroxyproline was tested for inhibition against *T. tonsurans* by the use of auxanograms, by addition to solid media, and by addition to liquid media. These experiments indicated only a very slight inhibition even at high concentrations (4.7 grams per liter).

Among the sixteen *T. tonsurans* strains tested, one amino acid-

deficient strain was found. Strain number 2 would not grow with inorganic nitrogen, and made no growth with amino acid mixtures lacking arginine. It did, however, produce a fair amount of growth when arginine was supplied as a sole source of nitrogen. It was found further that arginine could be substituted for by either citrulline or ornithine as sole sources of nitrogen respectively. This strain appeared therefore to be similar to a strain of *T. tonsurans* described by Wood (16) which was deficient for ornithine, citrulline, or arginine.

TABLE VI

GROWTH OF TRICHOPHYTON TONSURANS ON DIFFERENT SINGLE AMINO ACIDS

Amino acid	Colony diameter after 14 days Average of 5 strains
L arginine	22.0 mm
L ornithine	21.2 mm
L proline	15.4 mm
DL serine	15.4 mm
DL alanine	11.8 mm
L cystine	10.4 mm
DL phenylalanine	9.8 mm
Glycine	8.2 mm
L tyrosine (suspension)	8.0 mm
L leucine	7.8 mm
L tryptophane	7.6 mm (mycelium very thin)
DL valine	6.0 mm
L hydroxyproline	3.6 mm
DL isoleucine	2.6 mm
L lysine	2.6 mm
DL threonine	2.4 mm
L histidine	2.2 mm
DL methionine	2.2 mm
DL norleucine	2.2 mm
Control (no amino acid)	0.0 mm

Amino acid added in sufficient quantity to supply 0.2 gram of nitrogen per liter by L form.

To determine the frequency of occurrence of this amino acid-deficient variety and to correlate it, if possible, with some of the morphological variants, thirty-four more strains were surveyed. This was done by observing if isolates failed to grow on ammonium nitrate, since apparently the inability to grow on ammonium nitrate is associated with an amino acid deficiency. Those which failed to grow on ammonium nitrate medium were then tested for ability to grow with arginine, citrulline, or ornithine in media otherwise nitrogen free. By this method six more deficient strains were found and all were shown to be deficient for some component of the ornithine, citrulline, arginine cycle. This

made a total of seven amino acid-deficient strains detected in fifty studied. Of these, six had produced a yellow surface pigment and had been identified as *T. tonsurans* variety *sulfureum*. However, seven other *sulfureum* varieties when examined by the same method were not amino acid-deficient, as is shown in TABLE VII. Of the seven amino acid-deficient isolates, two grew on ornithine, citrulline, or arginine and five grew only on citrulline or arginine as sole sources of nitrogen (FIG. 4).

The control cultures, strains of *T. mentagrophytes* and *T. rubrum*, were similarly studied for amino acid utilization. In general, they reacted similarly to the *T. tonsurans* strains except that no amino acid-deficient strains were found.

Carbon utilization studies. Carbon studies were carried out by using ammonium nitrate as the nitrogen source and adding different carbon compounds at the rate of ten grams per liter. At pH 6.0 a limited

TABLE VII

SUMMARY OF TRICHOPHYTON STRAINS STUDIED AND SIGNIFICANT NUTRITIONAL CHARACTERISTICS FOUND

Strain number	History or source	Thiamine deficiency	Growth on nitrate	Amino acid deficiency
1	*T. tonsurans*—Dr. J. L. Pipkin, Texas	Yes	Yes	None
2	*T. tonsurans* (var. sulfureum)—New Jersey	Yes	No	Citrulline or arginine
3–9	*T. tonsurans*—Dr. J. L. Pipkin, Texas	Yes	Yes	None
10	*T. sulfureum*—Sabouraud Collection, Paris	Yes	Yes	None
11	*T. acuminatum*—Dr. A. Catanei, Algeria	Yes	Yes	None
12–14	Isolated from endothrix hair, C.D.C. Laboratory	Yes	Yes	None
15	Pleomorphic var. of Strain No. 7	Partial	Yes	None
16	Pleomorphic var. of Strain No. 9	Partial	Yes	None
17–29	*T. tonsurans*—Dr. J. W. Wilson, California	Yes	Yes	None
30	*T. tonsurans*—Dr. J. W. Wilson, California	Yes	No	Citrulline or arginine
31	*T. rotundum*—Sabouraud Collection, Paris	Yes	Yes	None
32	*T. crateriforme*—Sabouraud Collection, Paris	Yes	Yes	None
33	*T. tonsurans* (variety sulfureum)—Strain Shelmire, Dr. R. W. Benham, New York	Yes	No	Ornithine, citrulline or arginine
34	*T. plicatile*—Sabouraud Collection, Paris	Yes	Yes	None
35	*T. sulfureum*—Dr. A. C. Curtis, Michigan	Yes	No	Citrulline or arginine
36	*T. plicatile*—Strain Kurotchin, Dr. R. W. Benham, New York	Yes	Yes	None

TABLE VII—(*Continued*)

Strain number	History or source	Thiamine deficiency	Growth on nitrate	Amino acid deficiency
37	*T. tonsurans*—Vanderbilt Clinic, New York, Dr. R. W. Benham	Yes	Yes	None
38	*T. tonsurans*—Dr. H. Price, California	Yes	Yes	None
39	*T. sulfureum*—London School Hyg. Trop. Med.	Yes	Yes	None
40	*T. sulfureum*—London School Hyg. Trop. Med.	Yes	No	Citrulline or arginine
41	*T. sulfureum*—London School Hyg. Trop. Med.	Yes	Yes	None
42	*T. sulfureum*—London School Hyg. Trop. Med.	Yes	Yes	None
43	*T. sulfureum*—London School Hyg. Trop. Med.	Yes	Yes	None
44	*T. cerebriforme*—London School Hyg. Trop. Med.	Yes	Yes	None
45	*T. sulfureum*—London School Hyg. Trop. Med.	Yes	No	Citrulline or arginine
46	*T. tonsurans*—Dr. E. S. Keeping, Canada	Yes	Yes	None
47	*T. sulfureum*—same as strain No. 45	Yes	No	Ornithine, citrulline or arginine
48	*T. flavum*—Dr. A. Catanei, Algeria	Yes	Yes	None
49	*T. fumatum*—Dr. A. Catanei, Algeria	Yes	Yes	None
50	*T. umbilicatum*—Dr. A. Catanei, Algeria	Yes	Yes	None
	Other *Trichophyton* species carried as controls in these experiments:			
51–52	*T. rubrum*—recent isolate	No	Yes	None
53–54	*T. mentagrophytes*	No	Yes	None

amount of growth was possible in the presence of a suitable carbon source.

The following carbon sources were tested: D fructose, D glucose, D galactose, D mannose, L sorbose, D xylose, L arabinose, lactose, sucrose, cellobiose, maltose, trehalose, raffinose, glycerol, L rhamnose, salicin, glycogen, sodium actetate, and sodium citrate. As shown in TABLE VIII, the hexoses (glucose, mannose, fructose, and galactose) and the disaccharides (cellobiose and trehalose) supported the best growth. Lactose, xylose, sucrose, arabinose, glycerin, sodium acetate, sodium citrate, and raffinose supported little or no growth. Salicin, sorbose, and glycogen supported limited growth.

The pleomorphic *T. tonsurans* strains showed no significant difference when compared with the normal strains. There was no significant difference in the carbon utilization between the *T. tonsurans* strains studied and the *T. rubrum* and *T. mentagrophytes* strains that were included for comparison.

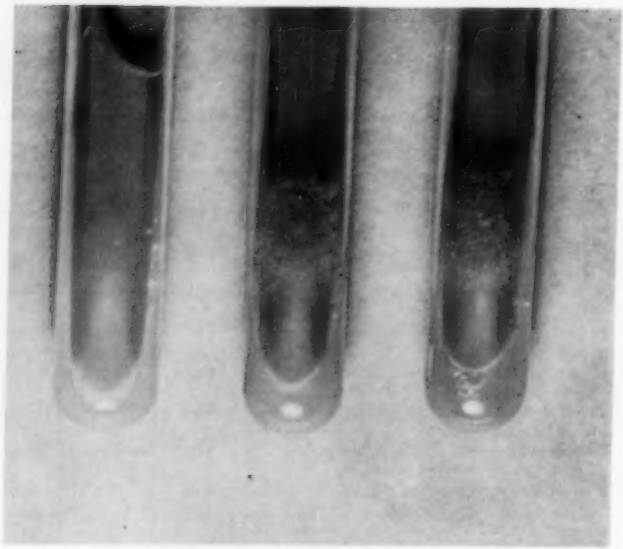

FIG. 4. *T. tonsurans* strain No. 2 growing on agar media using ornithine, citrulline, and arginine (left to right) as the sole sources of nitrogen. (Growth on ornithine is largely submerged, and is difficult to demonstrate by photography.)

TABLE VIII

GROWTH OF TRICHOPHYTON TONSURANS STRAIN NO. 6 ON DIFFERENT CARBON SOURCES

Class	Compound	28 Days' growth*
Pentose	D xylose	0
Pentose	L arabinose	0
Methyl Pentose	L rhamnose	0
Hexose	D glucose	2+
Hexose	D mannose	2+
Hexose	D fructose	2+
Hexose	D galactose	2+
Hexose	L sorbose	1+
Disaccharide	maltose	0
Disaccharide	cellobiose	2+
Disaccharide	lactose	0
Disaccharide	sucrose	0
Disaccharide	trehalose	2+
Trisaccharide	raffinose	0
Polysaccharide	glycogen	1+
Glucoside	salicin	1+
Acetate	sodium acetate	0
Citrate	sodium citrate	0
Trihydric alcohol	glycerin	0

* 0 No growth, 1+ poor growth, 2+ fair growth. Data given for one strain, as all other strains tested gave similar results.

DISCUSSION

These experiments indicate that *T. tonsurans* is greatly stimulated by thiamine. Fifty strains were tested and all responded by showing greatly increased growth in the presence of thiamine. Complete deficiency could not be shown, however, for any one strain, and indeed the amount of stimulation varied with the strain tested. The fact that the obviously pleomorphic strain showed considerable growth on a thiamine-deficient medium and that the normal, sporulating strains grew very poorly suggests that the degree of thiamine deficiency corresponds to the state of the culture. It may be that complete deficiency could not be shown simply because there is always a tendency towards pleomorphism in any strain. Another factor which may prevent the detection of a complete deficiency is that it is difficult to be certain the medium is completely free of thiamine. Titration experiments have shown that the sporulating form of *T. tonsurans* may be stimulated by thiamine at a concentration of as little as 0.000003 microgram in five ml of Sabouraud dextrose broth. Since *P. blakesleeanus* does not respond to less than 0.00003 microgram, when tested by similar methods, it can readily be seen that *T. tonsurans* is more sensitive than the organism used to test the purity of the thiamine-free medium. However, the magnitude of the response of *T. tonsurans* when grown in the presence of thiamine, as compared with growth on a thiamine-deficient medium, is quite sufficient to differentiate it from atypical strains of the nondeficient dermatophytes, *T. rubrum* and *T. mentagrophytes* (FIG. 5). For routine diagnostic purposes a simple test for thiamine stimulation may be performed by inoculating an unknown strain onto tubes of casein vitamin-free agar and a similar medium to which thiamine has been added. A decided difference in growth, with at least twice as much growth on the medium containing thiamine, indicates definite stimulation.

It has also been shown that the pyrimidine portion of the thiamine molecule is the effective moiety for this organism. Of the five pyrimidines tested, two supported good growth in the absence of thiamine. These two compounds have a methyl group in the 2 position and an amino group in the 4th or 6th position of the pyrimidine ring. Other studies have indicated that these are the critical positions for other organisms (**14**). The three pyrimidine compounds that did not support growth did not satisfy these conditions. Similar negative results have been obtained with 2,6-dihydroxy-4,5-diamino pyrimidine when tested with *Sporotrichum schenckii*, another organism requiring pyrimidine (**3**).

It is interesting that one other dermatophyte which shows a partial requirement for thiamine, *T. violaceum*, also responds to pyrimidine

(6). Tests with the various pyrimidines used in this study indicate that the same pyrimidine compounds are active for both *T. violaceum* and *T. tonsurans*. On the basis of studies that have been completed, it would appear, therefore, that nutrition tests would be of little value in differentiating these dermatophytes. Fortunately, however, although these two fungi produce similar disease clinically, their cultures are quite distinct morphologically and are not likely to be confused.

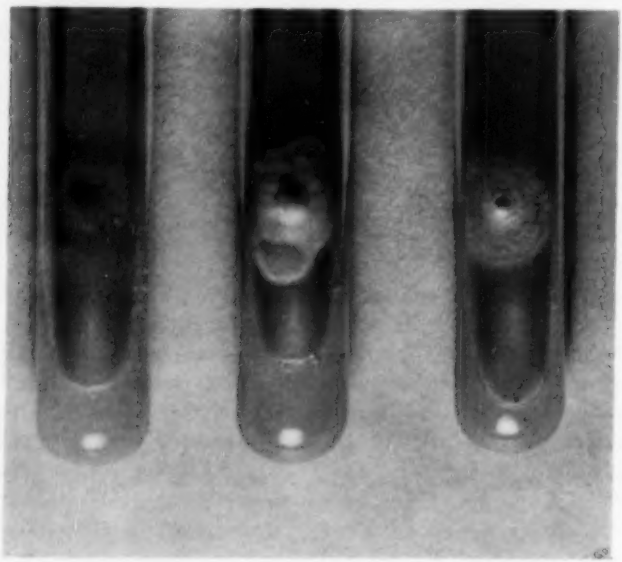

FIG. 5. *T. tonsurans*, *T. rubrum*, and *T. mentagrophytes* growing on vitamin-free casein agar medium.

With regard to nitrogen source, it has been shown that *T. tonsurans* grew considerably better when supplied with organic nitrogen compounds. However, it has been shown that by carefully controlling the pH a limited amount of growth is possible on ammonium nitrate. It seems likely that an unfavorable pH was responsible for the reports by other authors of lack of growth or very poor growth with inorganic nitrogen compounds. Tests with ammonium sulfate and sodium nitrate indicate that the ammonium ion is the nitrogen form utilized. Robbins (13) reported this to be true for *T. mentagrophytes*. However, Foster (5) has reported that utilization of ammonium or nitrate by some fungi is dependent upon pH.

Most strains of *T. tonsurans* do not have deficiencies for any amino acid. Occasionally, however, one is found that is deficient for a component of the ornithine, citrulline, arginine cycle. These can be easily detected by determining if growth does or does not occur with ammonium nitrate as the nitrogen source. The normal strains grow to a limited extent with ammonium nitrate as the nitrogen source, while the deficient organisms fail to grow. It seems probable that a change from one amino acid deficiency to another is easily made. That is, an amino acid-deficient strain able to utilize either ornithine, citrulline, or arginine as a sole source of nitrogen may lose the ability to utilize ornithine. Conversely, an amino acid-deficient strain able to utilize only citrulline or arginine as a sole source of nitrogen may gain the ability to utilize ornithine. It will be noted in TABLE VII that strains 45 and 47 were identical in origin, but subcultures were obtained from two different sources and both were inadvertently tested for amino acid deficiency. It can be seen that strain number 45 is different from strain number 47 in that it has lost the ability to grow on ornithine as a sole source of nitrogen.

It was found possible to test the assimilation of different carbohydrates by growing the test organism on a basal agar medium adjusted to pH 6.0 and containing ammonium nitrate to which different carbon compounds had been added. While it was found that there was no difference in the compounds utilized by *T. tonsurans, T. mentagrophytes,* and *T. rubrum,* some interesting results were obtained. Good growth occurred when the hexoses (dextrose, mannose, fructose and galactose), were present. The disaccharides (cellobiose and trehalose) were also well utilized. This demonstrated the ability to hydrolyze the alpha glucoside linkage (trehalose) and the beta glucoside linkage (cellobiose). However, maltose (alpha glucoside) and lactose (beta glucoside) were not utilized. Evidently, the enzymatic breakdown of these disaccharides is dependent upon the configuration of the entire molecule rather than just the type of linkage.

The possible value of these nutritional studies in the laboratory identification of *T. tonsurans* is suggested by the finding of a specific vitamin deficiency for this species, and the fact that this requirement for thiamine, or more specifically pyrimidine, is not characteristic of two other dermatophytes, *T. mentagrophytes* and *T. rubrum,* with which it might be confused on the basis of morphological studies. The fact that this deficiency is more difficult to demonstrate with the pleomorphic form of *T. tonsurans* does not lessen its value as a diagnostic tool, because this fungus ordinarily is isolated in the normal sporulating

form, and usually only old stock cultures show pleomorphism. The requirements for various members of the ornithine, arginine, citrulline cycle by certain strains of *T. tonsurans* is an interesting finding. However, such deficiencies appear to be labile and certainly are not characteristic of all isolates of this species.

The fact that a number of specifically named forms in this group: *T. crateriforme, T. sulfureum, T. umbilicatum, T. plicatile, T. rotundum, T. cerebriforme, T. acuminatum, T. flavum* and *T. fumatum*, concerning which there has been considerable controversy regarding their designation as species, all show similar partial deficiences for thiamine, gives further evidence that they are closely related and should be considered as simply morphological variants of *T. tonsurans*.

Practical applications of these nutritional studies in the identification of *T. tonsurans* in conjunction with morphological studies, with a review of the taxonomy of this organism, will be presented in succeeding papers, (8, 9).

SUMMARY

1. The sporulating form of *T. tonsurans* has been shown to require thiamine for good growth.

2. The pleomorphic (nonsporulating) form is less sensitive to the presence or absence of thiamine.

3. Thiamine may be substituted for by the pyrimidine moiety of the thiamine molecule.

4. In the presence of thiamine, *T. tonsurans* grows best with an organic nitrogen source, although some growth is possible with ammonium nitrate at pH 5.8–6.2.

5. Most isolates of *T. tonsurans* are not deficient for any one amino acid but L arginine, L. ornithine, L proline, DL serine, and DL alanine stimulated growth more than any of the other amino acids tested.

6. Seven of fifty strains of *T. tonsurans* tested were deficient for ornithine, citrulline, or arginine, or a combination of these.

7. Using ammonium nitrate as a nitrogen source, the utilization of various carbon compounds was tested. The hexoses (glucose, mannose, fructose, galactose) and the disaccharides (cellobiose and trehalose) supported the best growth.

8. Testing for stimulation by thiamine is suggested as an aid in the identification of morphologically atypical strains of *T. tonsurans*.

9. The fact that a number of named "species" in the so-called Crateriform Group show similar requirements for thiamine is further evidence for equating all these forms with *T. tonsurans*.

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FURTHER STUDIES RELATING TO DOMINANCE AND ASCUS ABORTION IN NEUROSPORA TETRASPERMA [1]

B. O. Dodge

(with 1 figure)

As an organism for biochemical-genetic study *Neurospora crassa* has no near rival, and it is most reassuring to see that the vast amount of data assembled by the biochemical geneticists working with this species has all been conserved and is being used in preparing detailed chromosome maps. That highly important field of genetics has necessarily been centered around the haploid stage of the fungus. The ascus, the only diploid cell in the life cycle, has, one might think, little to recommend it morphologically as a subject for genetic research. In some ways, however, it is the most interesting and essential structure of all if one considers what is going on, or may be prevented from going on, in that one diploid cell.

The writer and his associates have reported their cytological and genetic work on species of *Neurospora,* particularly on *Neurospora tetrasperma,* and have shown that other facultatively heterothallic species like *Gelasinospora tetrasperma* and *Pleurage anserina* furnish excellent material for genetic work. They have illustrated examples of dominance, recessiveness, dominant lethals, pleiotropic genes and phenocopies, using the ascus as the basic diploid structure. Published work describing these examples will be briefly mentioned, after which certain questions that still await solution will be raised. Finally, without giving the details respecting the culture work, the answers to these questions, so far as yet proved, will be given.

1. When *Neurospora tetrasperma* is cultured on certain media, asci that are heterozygous, *AaIi,* usually abort without delimiting any spores. They then become more or less dark colored, indurated and striated.

[1] The writer is indebted to Prof. Wm. J. Robbins, Director of The New York Botanical Garden, for the use of a number of special culture media in connection with our attempts to prevent abortion of homozygous asci, *AaEE.* Dr. Donald P. Rogers has read the manuscript and has made certain suggestions which have been accepted with appreciation.

This illustrates the effect of the dominant gene *I*. If such an ascus does not abort it usually cuts out four heterocaryotic spores, (*AI–ai*) or (*aI–Ai*). Cases of non-genetic indurated ascus abortion have been reported as occurring sporadically in cultures of *Neurospora*. Such chitinized aborted asci are true phenocopies of those proved to be due to gene *I* (**1**).

2. Asci heterozygous *AaDd* do not usually abort. They delimit four spores rather normally. Gene *d* is a recessive lethal for ascus abortion, *D* being the dominant wild type allele. Asci *Aadd* abort and deliquesce (**2**).

3. When the fungus is cultured on corn-meal agar, asci heterozygous *AaEe* usually abort without cutting out spores. Gene *E* here is acting as a dominant lethal. If any ascus does not abort the gene is then not dominant for ascus abortion, but it is usually dominant for eight-sporedness. When new-potato-steep-dextrose agar, NPDS, is used, there is little or no ascus abortion (**3, 6**). A more striking example of dominance in fungi would be difficult to find. It is true that each of the eight spores is haploid, but the number of spores is more or less predetermined when nuclear fusion occurs to make the ascus cell diploid. So, gene *E*, acting as a dominant lethal for the abortion of one ascus, and a dominant for eight-sporedness in some adjacent ascus, is a good example of a pleiotropic gene.

The three lethal genes mentioned above are not linked. Mating type genes *A/a* and genes *E* and *d* are all so near their respective centromeres that crossing-over is rather rare. There is some evidence that cross-overs involving gene *I* are not very rare. The effects of these three lethal genes have been noted mostly as they have acted independently. However, the writer and his associate, Bernice Seaver, made crosses that would bring together in the same ascus genes *I* and *d*. Asci that were homozygous for *d*, *AaIidd*, all aborted owing to the *dd* condition and then became indurated, striated and colored owing to gene *I* (**5**). Much later (**6**) it was discovered how to culture independently races *AE* and *aE*, but it was not yet known how to grow homocaryotic races *AI* and *aI*. With this accomplished, the next step would be to use genes *d*, *I* and *E* in attempts to make the following crosses: *AI* × *aI*, *AE* × *aE*, *AI* × *aE*, *AE* × *aI*, and *AED* × *aed* and *AEd* × *aed*. It can be reported here that all the above crosses have been made. The results prove that one could not very well forecast the pictures of behavior developed by the following types of asci: *AaeeII*, *AaiiEE*, *AaIiEe*, *AaEeDd* and *AaEedd*. The writer will report only what happened under the existing culture conditions and methods employed.

Other conditions—media, light, heat, etc.—no doubt would alter the pictures somewhat.

CULTURING INDEPENDENTLY "LETHAL" RACES AI AND aI

One of the side effects of a dominant lethal in maize is said to be manifested when the pollen spore dies. If it germinates the tube will not function in fertilization. The first thing to be done in order to produce certain of the crosses mentioned previously was to obtain parental races proved to be strictly homocaryotic *AI* and *aI*. This proved to be a rather tedious and exacting matter. A brief resumé of this work follows. When we first studied heterocaryotic races of *Neurospora tetrasperma* carrying gene *I* (1) it was noticed that certain small ascospores germinated well, but the germlings died. It was assumed, therefore, that no one need expect to grow in culture by itself a component race that carries gene *I* in all its nuclei. It was thought that the lethal must necessarily be accompanied by the normal *i* allele in nuclei in the same cytoplasm to provide a heterocaryon (*AI–ai*) or (*Ai–aI*). It was many years later, rather recently, in fact, that such an assumption was proved entirely false. For example, to quote from one record: "Apr. 11, 1952. At least 50 one-spore germlings from ascospores from a culture 481.3 . . . 7.29 were isolated. Several of the isolate cultures produced no perithecia. They were *good growers* so were not tested *as they could not have carried lethal I*. Some of these looked as though they might be *I, but they grew too fast.*" (I am italicising here some words to indicate our belief at that time.)

On Sept. 9, 1952, we isolated about 50 germlings that looked like *I* races. The next day we isolated 15 other germlings that were all "stunted." Of the 75 one-spore germlings that grew enough to be scored, forty-one produced perithecia with aborted asci. One produced perithecia showing four-spored asci but no aborted asci. Thirteen were not tested *because they grew and looked like normal i races.* From what was discovered more recently some of these might have proved to be pure lethals *AI* or *aI* if tested. As it turned out, Nos. 52, 61, 67 and 69 were proved to be good *aI* races. When grown on PDS agar masses of orange-colored conidia developed. Sub-cultures from race No. 52 have been tested many times in crosses with our tester 394.4 (= *Ai*). The resulting perithecia always contain many aborted asci when the Difco potato dextrose medium is used.

Race No. 74 was probably heterocaryotic originally, but by repeated sub-culturing the component *AI* was dissociated from its companion *ai*.

The *AI* culture was difficult to keep alive. It soon died in storage, so we had then only the four *aI* races, Nos. 52, 61, 67 and 69. The last two died after a few months in storage. Two races, Nos. 71 and 72, were clearly "doubles," *AI–aI*. They grew slowly yet finally produced a few perithecia with aborted asci. They also fruited fairly well when grown separately with each of the testers 394.14 and 394.5. These "double lethal" heterocaryons are the more interesting because they prove that it is possible to develop asci that are homozygous *AaII*. This point is being discussed in a later report.

More satisfactory results were obtained when 58 germlings were isolated Feb. 1, 1954. These were selected because it was thought the spores were small and, therefore, would have only a single nucleus originally. This nucleus would be either *Ai, ai, aI* or *AI*. Nineteen died early. Thirteen proved to be bisexual, developing perithecia with some aborted asci. When cultured on Difco PD agar most of the asci aborted, but one could usually find some scattered ascospores in crushed mounts of old perithecia. Four of the isolates were homocaryotic *Ai–ai*. Neither component carried factor *I*. Even so these four races were sub-normal in growth and in the production of fruit-bodies. There was no visible abortion of asci, yet the perithecia matured a greatly reduced number of four-spored asci such as would characterize normal perithecia of this species. One isolate, D-31, was proved to be a "double lethal," *AI–aI*. In case of a cross-over there could be an ascus with two spores *Ai–ai* ("normal") and two *AI–aI*. This was tested out by growing these "doubles" with normal testers *Ai* and *ai*.

Unpublished records of similar isolation work made in late 1934 indicate that such doubles *AI–aI* may not be rare. Sixteen germlings were chosen particularly because the ascospores were large, that is, normal in size for this species. The germlings were very abnormal in appearance, and were slow-growing. None of them lived to make readily visible mycelia. They were all finally removed from the tubes and measured. The average length of the spores was 32 μ, the largest being 37 μ and the smallest 29 μ. No doubt they were all *AI–aI*. If any had been either *AI–ai* or *Ai–aI*, it would have grown vigorously and fruited.

Seven of the 58 germlings, isolated as noted above, Nos. D-8, D-11, D-14, D-15, D-25, D-26 and D-34, were proved to be *aI*, and, best of all, three, D-2, D-4 and D-6, were found to be *AI*. This gave us two kinds of homocaryotic races, *AI* and *aI*, that can be used independently in making the various types of crosses mentioned earlier.

DISSOCIATION OF COMPONENTS OF HETEROCARYON 481.3 . . . 7

The following report concerns the lethal *I* and normal *i* components of the heterocaryotic race 481.3 . . . 7, and their essential characters and mating relations when dissociated and cultured separately as homocaryons that could be used for breeding purposes. The primary reason for starting this work on dissociation was, however, to see if, during the time this race had been kept in stock culture, it might have suffered changes as a result of somatic mutations, reversions or contamination. The particular material employed was a culture that Dr. W. J. Robbins maintains for some of his physiological and biochemical investigations.

The marked feature of race 481.3 . . . 7 is that all the nuclei of one of its mating-type components carry the dominant *I* for ascus abortion while all the nuclei of the opposite mating type carry the recessive *i* gene. Any asci developed in mature perithecia will be heterozygous *AaIi*. When cultured on Difco potato-dextrose agar, for example, the asci will usually abort characteristically without spore formation (1). This race represents the tenth filial generation carried for twenty years in cultures and propagated at intervals from ascospore to ascospore, each spore being either (*AI–ai*) or (*Ai–aI*). It should be noted that during this long period, much of the time in cold storage, the lethal effect of *I* in causing ascus abortion on corn-meal agar seems to have weakened. We now must rely more or less on Difco potato-dextrose agar for detecting the presence of lethal *I* in mating effects. A potato-dextrose made with a steep from old potatoes also serves very well. It may be that our success in culturing lethal races *AI* and *aI* as homocaryons owes something to a further weakening of gene *I* in its power to kill germlings carrying it. It will now be shown how one can dissociate and culture independently the two complementary components of race 481.3 . . . 7.

On October 27, 1953, conidia were spread thinly on two plates of PD agar and two plates of PDS medium. They germinated quickly on both kinds of media. The next day 85 one-conidium germlings were transferred to small tubes of PD, BM and BM + PE media in about equal numbers. The particular germlings were chosen because they were, in general, slow-growing and came from the smaller conidia. By November 5th it was found that ten germling isolates on BM and three on PD had died without further growth. The larger, more vigorously growing germlings would, no doubt, have been bisexual or heterocaryotic exactly like race 481.3 . . . 7 itself. By this time, many perithecia could be seen in several of the cultures. Mycelium from twelve

other cultures that appeared to be abnormal and still sterile (unisexual) was transferred to twelve tubes of PD in duplicate. They were then all tested for mating types *A* and *a* and also for lethal *I* and *i*, using stock cultures 394.4 (= *Ai*) and 394.5 (= *ai*) as testers. The results could not be determined until after December 22nd. It had been necessary to have these test-mating cultures placed in cold storage after they had been in a 27° C incubator for three weeks. It was now (Dec. 23) found that isolates H-3, H-4, H-6, H-7, H-10, H-11, and H-12 had all fruited well with tester *Ai*, but not at all with tester *ai*. Since many aborted asci could always be found in perithecia developed in these test cultures, it was clear that all seven were of the same genotype *aI*. Four other isolates H-1, H-2, H-5, and H-9 all fruited with tester 394.5 (= *ai*). The perithecia that had been developing very slowly and sparsely were examined several times. The asci that did finally mature had four spores. In none of these test cultures could one find the type of ascus abortion that indicates the presence of the lethal *I* gene. The four isolates were of the genotype *Ai*, non-lethal and normal so far as an *I* gene was concerned, but subnormal and of low fertility in test matings. It can be said, therefore, that the two components of the heterocaryon 481.3 . . . 7 are *Ai* and *aI*. All the *Ai* isolates are exactly alike, and all the *aI* isolates are alike, since all *Ai* isolates are merely "cuttings" from the *Ai* component clone as the *aI* isolates are cuttings from the *aI* component clone. It was proved that one could reconstitute the heterocaryon by growing the component *Ai* in culture with component *aI*. It does not matter which particular isolates are chosen so long as an *Ai* is grown against an *aI*. Isolate H-8 proved to be bisexual. The perithecia developed many aborted asci. Twenty-four other isolates were not designated by number. They were all bisexual and their perithecia developed many aborted asci. They must all have been just like 481.3 . . . 7.

There were in all 46 one-conidium isolates that proved to be unisexual or self-sterile and homocaryotic. Six of these were proved to be *Ai* and 40 were *aI*. Whether or not the components of other heterocaryons such as (*AI–ai*) derived from ascospores of a different generation could have been as easily separated and cultured independently, is a point that should be investigated.

After these 46 subcultures had grown on a PDS medium a few days they were laid out on a table. Very striking differences could be discerned by any observer. Isolates H-1, H-2, H-5, H-9, H-32 and H-36 showed only a few pale conidia and a grayish or light-brown aerial growth with little or no orange coloration. The other forty isolate

cultures showed more rapid growth, a heavier, bright-yellow mycelium comprising the growth on the slant and the masses of orange-colored conidia at the top. All these isolates were tested over and over against our testers in the expectation of finding some error or some gene interference to account for the lack of normal vigor of growth of *Ai* isolates which were so subnormal when mated. The forty *aI* isolates would certainly be mistaken for normal wild types in cultures on PDS even by those familiar with *Neurospora*.

This simple piece of culture work on dissociating the two components of the heterocaryon turned out to be very informative, as it has served to correct some of our early assumptions regarding the "dominant lethal"

TABLE I

DISSOCIATION OF HOMOCARYOTIC COMPONENT RACES OF THE HETEROCARYON 481.3 . . . 7

Summary of Results

Medium	BM Basal Medium	PE+BM Potato Ext. +BM	PD Difco Potato Dext.
Isolate lived	25	28	19
Isolate died	10	0	3
Genotype *Ai*	0	1	5
Genotype *aI*	9	16	15
Bisexual	6*	11**	9***

* The bisexual isolates on BM finally developed perithecia, but none of these developed any asci.

** All but one of these bisexual isolates developed perithecia with mostly aborted asci; that one grew rapidly from the first and showed young perithecia on the second day. Many asci were delimiting four spores within a week. Its phenotype would suggest a perfectly normal wild-type *Ai-ai* heterocaryon. It could have been either a contaminant or, less likely, a somatic reversion, *Ai* to *ai*.

*** On Difco PD agar a high percentage of the asci at first showed the "catfish" type of abortion, then deliquesced without becoming chitinized or indurated.

gene *I*. The highly colored and fairly rapid growth of these *aI* isolates explains why we were led astray in our many attempts to obtain cultures from small *AI* and *aI* ascospores. Previously in this paper it is reported how cultures of *aI* races were first obtained from ascospores, but race No. 74, thought to have been pure *AI*, died soon after being placed in storage. The fact stands out that, to date, it seems to be much easier to secure in culture *aI* races than it is to obtain and keep *AI* races growing. It might be fruitful to dissociate the components of a number of lethal *I*-bearing heterocaryons, some of which are bound to be *AI–ai* in their make-up. In fact Dodge and Seaver (**5**, p. 161) reported that of the 109 heterocaryons which they obtained from single ascospores, 55 were (*AI–ai*) and 54 were (*Ai–aI*). When one grows together in a culture, for example, isolate H-9 (= *Ai*) and isolate H-6

(= *aI*) there will be nuclear migrations to reconstruct the original heterocaryon 481.3 . . . *7*, after which perithecia with aborted asci will be developed. As stated previously, the Difco potato-dextrose medium should be used to bring on much abortion.

Having proved that our race 481.3 . . . *7* was a heterocaryon from which the "lethal" *aI* could easily be dissociated from the *Ai* component, we decided to germinate some of the small f_1 ascospores in further attempts to obtain a few more *AI* races. For this work a culture growing on a special BM + PE medium was made available. The perithecia were discharging ascospores in great profusion. By our usual methods about fifty one-spore germlings were isolated and grown on various media. The results were rather disappointing. Twenty-three isolates were found to be bisexual heterocaryons, *I–i*, which developed perithecia with aborted asci when grown on PD agar. Three were bisexual homocaryons *AI–aI*, but they finally died. Five produced no perithecia when grown with either tester. Some would call them "neutrals." Further tests on various media should have been made. Eleven isolates were *aI*, but only one, No. D-75, was doubtfully *AI*. Much more isolation work must be done before one can say that races *AI* are more lethal as killers or germlings than are races *aI*.

DEVELOPMENT OF ASCI HOMOZYGOUS AaiiEE

Dodge, Singleton and Rolnik (**6**) isolated and cultured thirty-one pure homocaryotic races of *N. tetrasperma* that carried this gene *E* either as *AE* or *aE*. By mating these races with the appropriate tester, *Ae* or *ae*, they found that the asci, all *AaEe*, either aborted or delimited spores, depending on the medium used. If the asci did not abort they cut out spores, eight in some asci, or seven or six in others. It will be shown very clearly later on that when certain *aE* races were mated with tester 394.4 (= *Ae*) many perithecia would mature more asci with only *four* spores than with *eight* spores. However, there are always some eight-spored asci in the same ascus rosette (FIG. 1, *D*). The reason for this is not yet clear. In the paper cited above brief mention was made of attempts to obtain fruitful perithecia when one parent was *AE* and the other was *aE*. About ninety cross matings, clones *AE* with clones *aE*, were made, but only a few of the cultures developed perithecia of size and character to prove that they actually represented young perithecia and not just protoperithecia. In no case could asci be found, and none of the perithecia showed ostiolar growth. It was noted that if one could find some medium or condition inducing the maturing of asci *AaEE* that

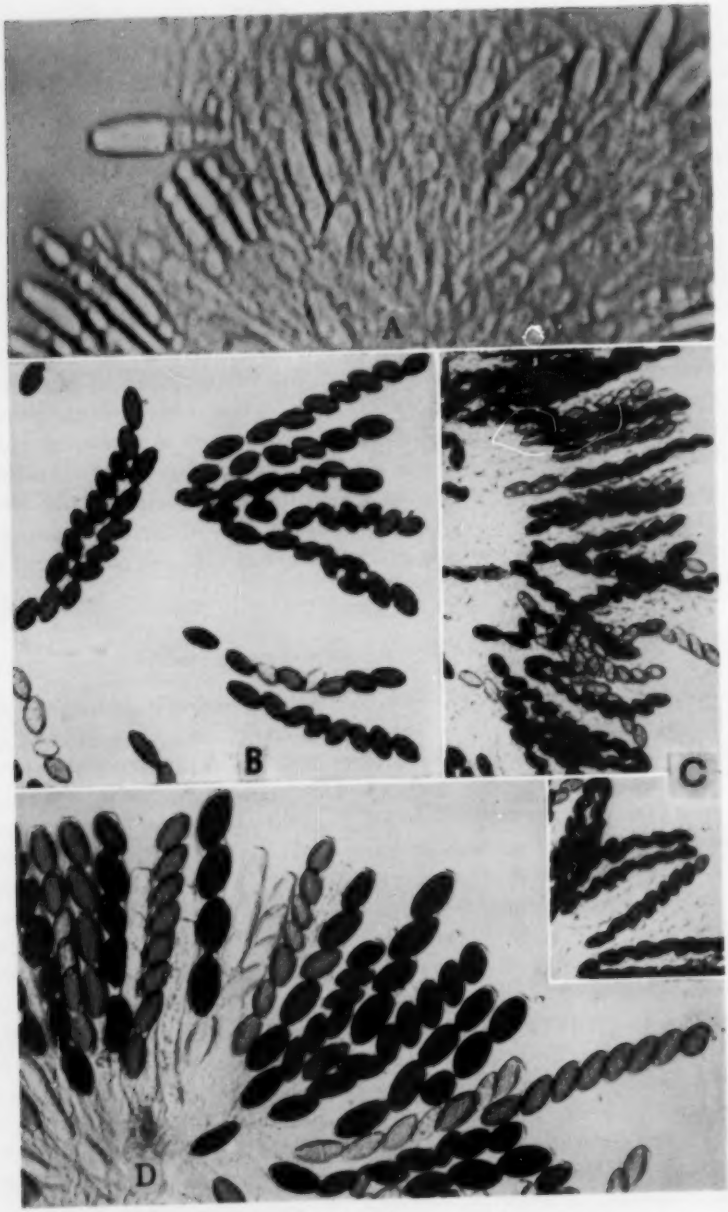

FIG. 1.

would not abort but continue on to delimit spores rather regularly, one could by close inbreeding establish races of *N. tetrasperma* with mostly eight-spored asci. No further attempts to continue that work were made until the summer of 1954.

Of the thirty-one races originally cultured only eight of each mating type, *AE* and *aE*, are kept in stock. Fresh culture transfers of each of these had been made early in this year and each was tested to prove its purity. Each culture was examined for the presence of protoperithecia. Races 343.6, 351.4, 325.5, all *AE*, and races 387.5, 381.5, 340.6 and 254.6, all *aE*, showed at least some good protoperithecia. Eventually each race *AE* was cultured with each race *aE* with various results. Where races 340.6 and 254.6 had been mated with either 343.6, 351.4 or 325.5, many black perithecia developed quickly in the cultures. Numbers of these perithecia formed the ostiolar papilla and in mounts one could see very definitely rosettes of aborting asci (FIG. 1, *A*). Some of the other matings developed the first stages of perithecial growth, but no asci were ever found in such cultures. Still other matings failed to show any perithecial structures at all. All these matings were repeated, some of them many times, using different media, with no better results. The combination 343.6 × 340.6 on a new-potato-steep-dextrose medium was very fruitful. Many asci developed rather well at first but, without exception, the asci finally aborted and deliquesced. Robbins's basal medium plus his potato extract, BM + PE, was especially good. Cultures produced vast numbers of black perithecia with many asci, but never any ascospores.

The mating 343.6 + 340.6 represents merely a mixed culture in which the nuclei from both races, by migration no doubt, came together to provide a heterocaryon (*AE–aE*). When one uses this combination (*AE–aE*) as one parent where the other parent is a normal tester, either *Ae* or *ae*, two or even three kinds of perithecia could be obtained. That is, the combination, or components, 343.6 + 340.6, of the mixed

FIG. 1, *A–D*. Portions of ascus rosettes of *N. tetrasperma*, magnifications various. See text for further explanations. *A*. All asci were homozygous, *AaEE;* all aborted without spore formation. The two parent races were 343.6 (= *AE*) and 340.6 (= *aE*). *B*. All asci were heterozygous, *AaEe*. In this case races 343.6, 340.6 and 394.5 (= *ae*) were grown together as a mixed culture in which only races 343.6 and 394.5 mated. No perithecia with aborted asci were developed in this culture. *C*. The same as *B* to show the predominance of eight-spored asci, much less magnification. *D*. Asci all heterozygous, *AaEe*, the same as in *B* and *C* but the mixture was 343.6, 340.6 and 394.4 (= *Ae*). Here the normal tester *Ae* mated with 340.6. The result proves that the "dominant" *E* gene may sometimes act as a recessive so that the ascus cuts out four spores.

culture, might mate regardless of the presence of the *Ae* or *ae*, to result in perithecia in which all asci aborted. But when the combination of races is (343.6 + 340.6) + 394.4 (= *Ae*), then the normal *Ae* mates with *aE* to form heterozygous asci *AaEe*. Or again, if the combination of races is (343.6 + 340.6) + 394.5 which is genotypically (*AE* + *aE*) + *ae*, and *ae* mates with *AE*, the asci would be the same as before, all *AaEe*. In either case such asci do not abort when the new-potato-steep-dextrose agar is used. The results of such combination matings show, however, that, considering the number of spores delimited in individual asci, the results need not be the same when race 340.6 (= *aE*) mates with race 394.4 (= *Ae*) as when 343.6 (= *AE*) mates with 394.5 (= *ae*) although the zygotes are all *AaEe*. This fact is brought out in FIG. 1. The partial rosettes of asci in *B* and *C* show mostly eight-spored asci, while in *D* the number of four-spored asci may equal the number of eight-spored asci.

It is clear that while asci heterozygous *AaEe* all cut out spores when one uses a new-potato-steep-dextrose agar, asci that are homozygous *AaEE* invariably abort without spore formation regardless of the kind of medium used. Furthermore, only when certain particularly favorable parental races of *AE* and *aE* are mated, are perithecia developed far enough to form asci. No medium that has as yet been tried enables gene *E* to operate effectively as a dominant for eight-sporedness in homozygous asci *AaEE* to prevent abortion and therefore enable the asci to delimit spores. No one could have predicted with certainty which of the two effects of this pleiotropic gene *E* would prevail in homozygous asci. Will it prevail in double force to cause total abortion of all asci, or will it prevail so that the ascus cuts out eight spores regularly? As indicated above, the double effect as a factor for abortion prevails perfectly over the double effect of this factor for eight-sporedness.

In our earlier paper (**6**) is found, p. 39, the statement, "The degree of dominance of *E* over *e* to regulate the number of spores delimited in these [*AaEe*] asci apparently is not changed by altering the medium." It can now be said that the number of such asci, *AaEe*, that delimit fewer than eight spores (seven, six, five or four) depends very much on the nature of the particular races mated. The results of the few preliminary tests seem to indicate that there may be some closely linked genes or factors that enter the situation to weaken the effect of gene *E* as a dominant for eight-sporedness over four-sporedness. It has been shown many times (**1, 5**) that gene *I* varies greatly in its dominance to cause ascus abortion when one alters the culture conditions.

The third report on work involving the "lethal" genes *I*, *d* and *E*

and their effects either singly or together in heterozygous and homozygous types of asci mentioned in the introduction, is now being prepared for publication. This will contain a further discussion and summary.

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TRICHOPHYTON MENTAGROPHYTES ISOLATED FROM THE SOIL OF CAVES

H. I. Lurie and R. Borok

(with 4 figures)

In 1952 Gordon *et al.* (2) isolated *Microsporum gypseum* from a random sample of soil in Williamson County, Tenn. Ajello (1), in testing a new technique for the isolation of dermatophytes from soil, also recovered this fungus from numerous soil samples in Williamson County. Zeidberg and Ajello (4), comparing the ecology of *H. capsulatum* and *M. gypseum*, found that while the former occurred predominantly in soils obtained from chicken houses and chicken yards, the latter was present chiefly in soils from barns, barnyards and around dwellings where animals are apt to be concentrated. They attributed this to the keratinophilic character of the dermatophyte.

During the past 2 years Murray [1] has been investigating cases of "cave disease" in a group of spelaeologists, the majority of whom showed a positive Histoplasmin skin test. During the course of this study samples of soil from caves frequented by the people affected were examined for the presence of *H. capsulatum*. Several techniques were employed, including the use of a membrane filter as described by Gordon and Cupp (3), biological and cultural methods.

On one of the filter membranes a macroconidium indistinguishable from those of *M. gypseum* was seen. This fungus was not recovered on culture.

Quantities of soil were suspended in saline and then allowed to stand for about an hour. The supernatant fluid was injected into mice and also plated on a variety of media. After 3 weeks the mice were killed and the viscera cultured. From two samples of soil *T. mentagrophytes* was isolated both from the mice viscera and from the direct culture. These samples were derived from Johnson's pothole, which is 60 feet deep, and from one of the Makapan caves at a distance of approximately 800 yards from the entrance. Both caves are situated in the Transvaal, are uninhabited and both are infested with bats.

[1] Personal communication from J. F. Murray.

The macroscopic and microscopic features of the fungus isolated correspond with those of *T. mentagrophytes* (*asteroides* type) (FIGS. 1, 2). It is pathogenic to guinea pigs. One pig was inoculated by rubbing a spore suspension into a scarification on the abdominal wall and another by intradermal injection. The former developed a small area of alopecia after 3 weeks. One week later the lesion had increased in size and

FIG. 1. Culture of fungus isolated from the soil of a cave. Dextrose agar, room temperature.

simulated a weeping eczema. The hairs had broken off level with the surface of the skin. Direct examination of epithelial scales showed the presence of septate, branching hyphae. The second pig showed a small scaly lesion after 15 days. A specimen was obtained for cultural examination and the animal was killed. The lesion was excised, fixed in formalin and sectioned. The sections showed the presence of hyperkeratosis, marked acanthosis and a slight perivascular round cell infiltrate

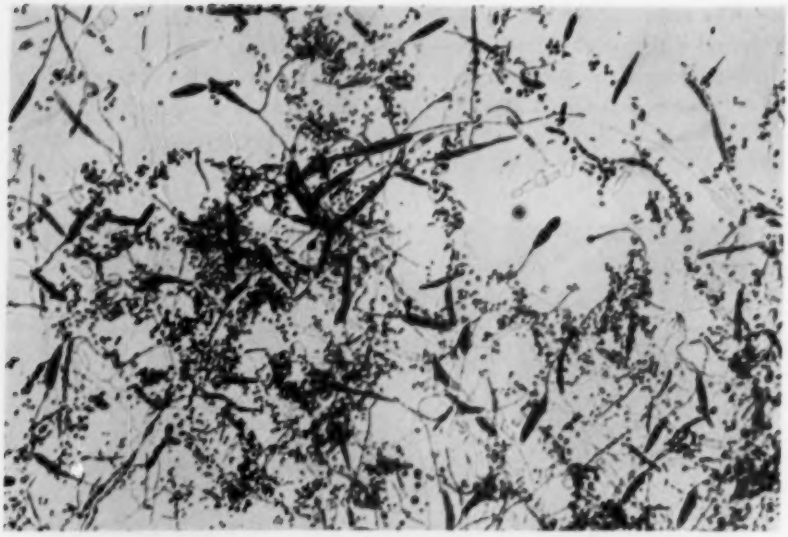

FIG. 2. Slide culture of fungus isolated from the soil of a cave. Numerous microconidia and macroconidia.

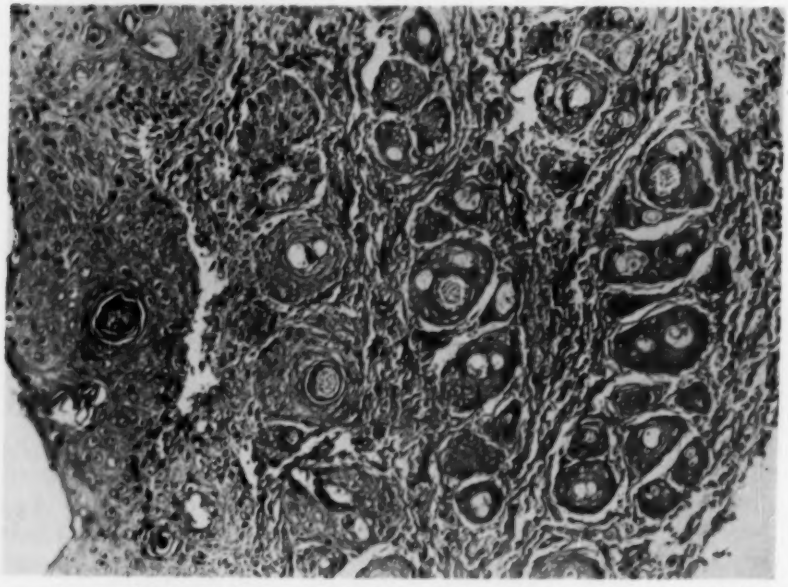

FIG. 3. Section of skin of guinea pig fifteen days after intradermal inoculation with spare suspension. Acanthosis of epidermis and slight round cells infiltrate in corium, × 100.

(FIG. 3). Periodic acid-Schiff stain revealed the presence of numerous microconidia in the follicles surrounding the hair shafts (FIG. 4). Retrocultures from both pigs were positive.

We believe that this is the first record of the isolation of *T. mentagrophytes* from soil. It is difficult to imagine how and when this fungus was introduced into these caves, as it is certainly several decades since they were inhabited by man or beast. During the examination of many

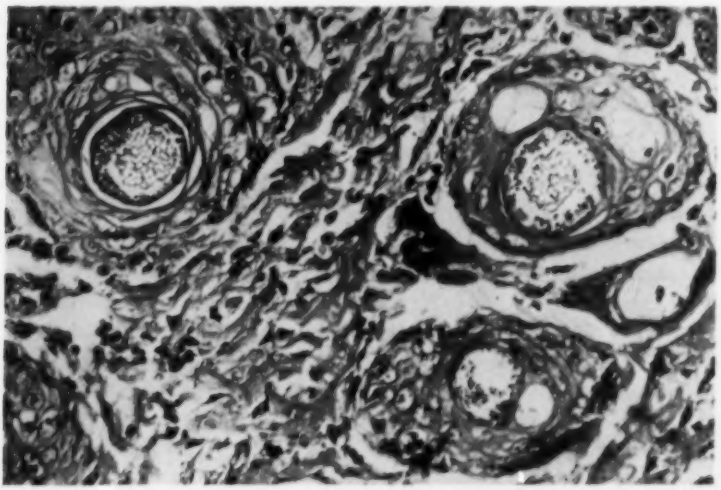

FIG. 4. Section of skin of guinea pig, × 350. Numerous small spores in hair follicles surrounding hair shafts.

bats from the Makapan cave for the presence of *H. capsulatum*, no skin lesions were noted.

SUMMARY

1. During a mycological examination of soil from caves in the Transvaal, a macroconidium resembling *M. gypseum* was seen in one sample.

2. *T. mentagrophytes* (*asteroides* type) was isolated from the soil of two caves.

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THE ASCOSTROMATIC ASCOMYCETES[1]

E. S. Luttrell

(with 2 figures)

In a previous review (Luttrell, **1951**) a summary of the literature on the classification of the Pyrenomycetes showed that, beginning with the establishment of the Dothideaceae (Fuckel, **1870**) and continuing through the work of von Hoehnel (**1907**, **1909**), Theissen and Sydow (**1918**), Arnaud (**1925**), Miller (**1928**, **1938**, **1949**), and Nannfeldt (**1932**), there has been increasing recognition of a distinct series of Ascomycetes in which the asci are produced in locules in an ascostroma. Despite that fact that the majority of species in this series were originally included in the same orders or even genera with perithecial forms, the evidence indicates that they have not been derived from the groups of Pyrenomycetes with which they have been classified but represent an entirely separate line of development. The chief orders in the ascostromatic series are the Myriangiales, Dothideales, and Pseudosphaeriales. This group was first assembled as the order Dothideales sensu ampl. by von Hoehnel (**1909**). Theissen and Sydow (**1918**) applied the name Dothidiineae to essentially the same group. This group, with the addition of the Hemisphaeriales, Hysteriales, and other elements, was first clearly defined and recognized as a distinct series by Nannfeldt (**1932**) under the name Ascoloculares. Luttrell (**1951**) placed primary emphasis on the bitunicate ascus as the fundamental characteristic of species in this series, to which he gave the name Bitunicatae.

Although various names have been applied to the ascostromatic series, none has been given definite taxonomic rank. It is proposed, therefore, that this series be recognized as a new subclass in the Ascomycetes. The first name suggested for this group as a distinct series is Nannfeldt's (**1932**) Ascoloculares. This is the most widely accepted name for this group, and it also is descriptive of one of the primary characteristics of the species which compose it. Consequently, with a change in form to make it correspond to other subclass names, it is used for the new subclass, which is described as follows:

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LOCULOASCOMYCETES subclass. nov.

Ascis bitunicatis, in ascostromate evolutis.

The composition and relationships of the Loculoascomycetes are shown diagrammatically in FIGS. 1 and 2.

The reasons for considering the Loculoascomycetes distinct from the true Pyrenomycetes have been given previously (Luttrell, **1951**). The separation is based primarily on the fact that with the ascostromatic nature of the ascocarp is correlated the production of bitunicate or two-walled asci. It is possible that ascostromatic forms could be derived from true Pyrenomycetes at many points by reduction of perithecial walls in forms in which the perithecia develop within a stroma, and this process probably has occurred—for example, in the Coryneliales and Coronophorales. However, in order to derive the species in the Loculoascomycetes from the Pyrenomycetes by this process, it is necessary to assume that the bitunicate ascus also developed at numerous points and coincidentally with the loss of perithecial walls. This seems too great a coincidence to be credible. It seems more probable that the species with bitunicate asci represent a separate monophyletic line. This conclusion is expressed in the erection of the subclass Loculoascomycetes. The primary characteristic of this subclass is the production of bitunicate asci. The ascostromatic nature of the ascocarp is a secondary correlated character.

The objections to this taxonomic hypothesis noted previously (Luttrell, **1951**) now seem less serious. These were the apparent lack of correlation between the production of bitunicate asci and ascostromatic ascocarps in several species and groups. First was the occurrence of bitunicate asci in forms considered to be Discomycetes such as the Hysteriaceae, *Keithia juniperi* J. K. Miller, and species of *Lecanidion* and *Tryblidiella*. A subsequent study (Luttrell, **1953**) showed that the ascocarp in *Glonium stellatum* Mühl. ex Fr., and presumably in other members of the Hysteriaceae, is an ascostroma. Pantidou and Korf (**1954**) found that the formation of an ascostromatic ascocarp is correlated with the production of bitunicate asci in *K. juniperi*. This species does not belong in the Discomycete genus *Keithia* but is synonymous with *Coccodothis sphaeroidea* (Cke.) Theiss. & Syd., a species which has been included among the ascostromatic forms in the Pseudosphaeriales. The report of Pantidou and Korf is of especial importance, since in this case the presence of the bitunicate ascus served as a valid indication of the correct taxonomic position of a fungus which had previously been misinterpreted. A study of species in the Patellariaceae with

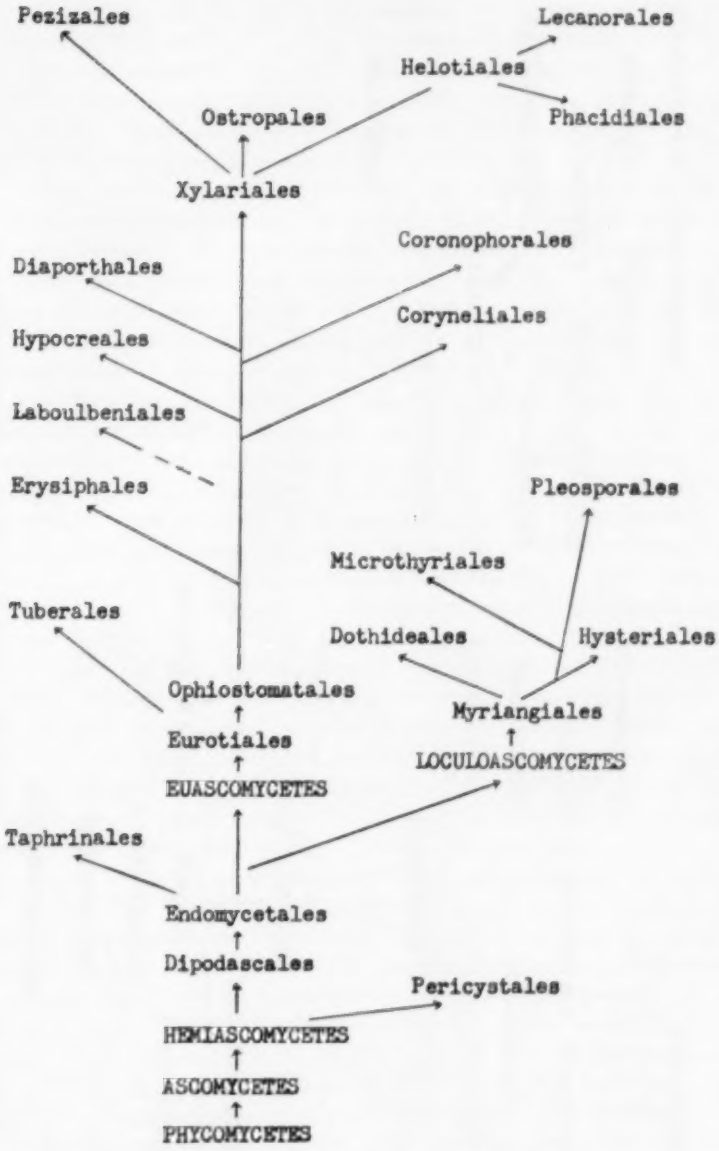

FIG. 1. Phylogenetic diagram of the Ascomycetes.

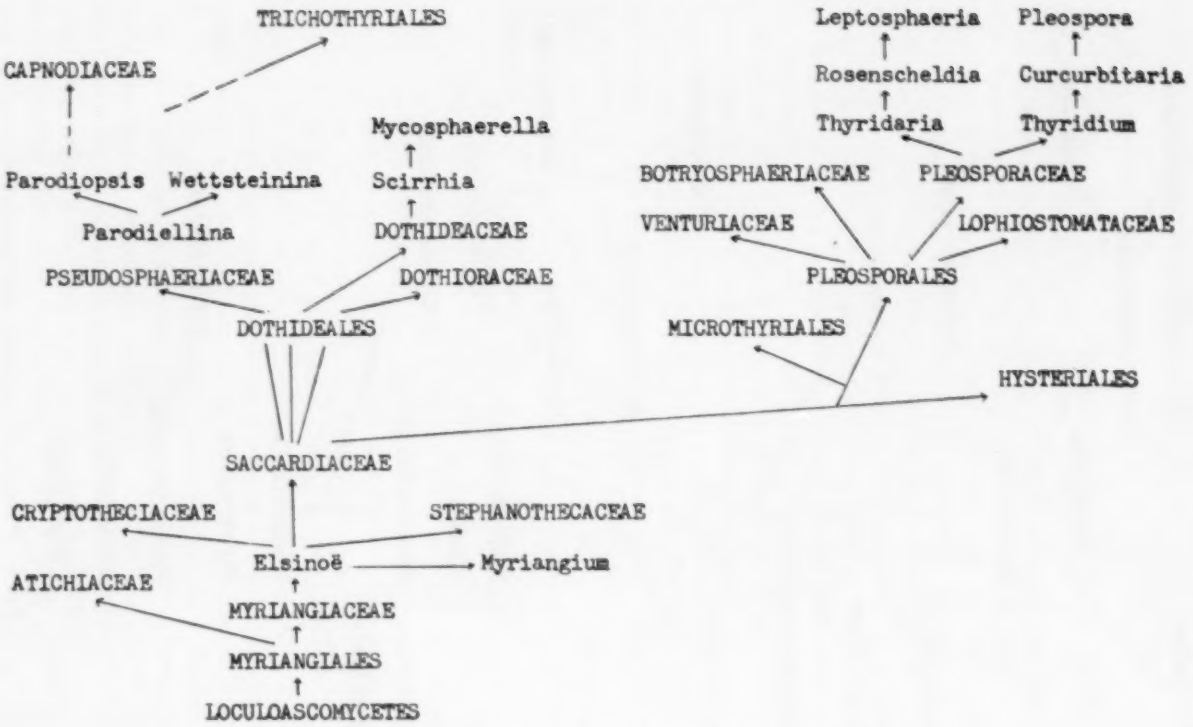

FIG. 2. Phylogenetic diagram of the subclass Loculoascomycetes.

bitunicate asci, such as those in *Lecanidion*, *Triblidiella*, and *Johansonia* (Müller, **1954**), might produce similar results. The transfers of *Coccodothis* back and forth from the Patellariaceae to the Pseudosphaeriales (Müller and Sanwal, **1954**; Petrak, **1954**) emphasize the importance of developmental studies to determine the nature of the ascocarp in species of these genera.

A second point was the formation of ascostromatic ascocarps in species with unitunicate asci, particularly those in the Coryneliales and Coronophorales. This was clearly indicated in the Coryneliaceae by McCormack's (**1936**) study of *Caliciopsis pinea* Pk. The Coronophorales are now better known through the taxonomic work of Munk (**1953**) and von Arx and Müller (**1954**) and Luc's (**1952**) developmental study of *Bertia moriformis* (Tode ex Fr.) de Not. Although Luc's study was incomplete, the figures indicate that the thin-walled, long-stipitate asci of *B. moriformis* are unitunicate and the ascocarp is an ascostroma. As mentioned previously, it is quite possible that an ascostroma could be produced by reduction of the perithecial walls in stromatic Pyrenomycetes. This process apparently has occurred in the Coryneliales and Coronophorales. The members of these orders have arrived at an ascostromatic structure resembling that of the Loculoascomycetes, but by a different path, being derived from perithecial forms in the Pyrenomycetes. They are definitely excluded from the Loculoascomycetes by their unitunicate asci. It is significant that Nannfeldt (**1932**), without mentioning the occurrence of unitunicate asci as the reason, perhaps intuitively excluded the Coronophorales from his Ascoloculares and allied them with the true Pyrenomycetes (Ascohymeniales). Munk (**1953**) also placed the Coronophorales among the Ascohymeniales. Von Arx and Müller (**1954**) likewise excluded this order from the Ascoloculares, placing it in the Plectomycetes.

So far as names are concerned, it seems necessary only to designate the group with bitunicate asci separated out of the Euascomycetes as the Loculoascomycetes. The remaining species continue to be designated Euascomycetes. There is no necessity for the name Unitunicatae (Luttrell, **1951**) since it is synonymous with the Euascomycetes in this limited sense. Within the Euascomycetes the classic names Plectomycetes, Pyrenomycetes, and Discomycetes may be retained for convenience of reference, although they have no definite status as taxonomic categories. There is no advantage in substituting Nannfeldt's (**1932**) names Plectascales and Ascohymeniales (true Pyrenomycetes and Discomycetes) since the division between the Plectomycetes and Pyrenomycetes is certainly no clearer than that between the Pyrenomycetes and Disco-

mycetes. Also, the ordinal endings of Nannfeldt's names may be a source of confusion.

CLASSIFICATION

The following arrangement of orders and families indicates the composition of the Loculoascomycetes. Unless noted otherwise the orders and families are as defined by Luttrell (**1951**).

Subclass III. LOCULOASCOMYCETES
Syn. ASCOLOCULARES, BITUNICATAE
Order I. MYRIANGIALES
Family 1. Atichiaceae
Family 2. Myriangiaceae
Syn. Elsinoaceae, Plectodiscellaceae
Family 3. Saccardiaceae
Family 4. Cryptotheciaceae

The Cryptotheciaceae includes the lichen genera *Cryptothecia* and *Stirtonia*, and possibly *Helminthocarpon* (Santesson, **1952**), which appear to be Myriangiaceous in structure. The globose, thick-walled asci arise singly in the effuse, crustose thallus and are separated by a loose thallus tissue. They may be scattered individually throughout the thallus or more or less closely grouped in differentiated fertile areas which, when highly developed, resemble the ascocarps of *Arthonia* spp. Santesson (**1945**) described bitunicate asci in *Cryptothecia epiphylla* R. Sant. These asci were of the unusual type with thick outer walls and relatively thin inner walls which has been reported previously only in *Myriangium* spp. (Luttrell, **1951**, p. 27). Santesson (**1945**) stated that there is no indication of a type of development similar to that in *Myriangium* as described by Miller (**1938**) and that the Cryptotheciaceae and Myriangiales are not related. As has been pointed out previously (Luttrell, **1951**), species of *Myriangium* form a highly developed branch of the Myriangiales and are not themselves representative of the basic developmental type of the order. The Cryptotheciaceae appear to be similar in structure to *Elsinoë* and *Atichia* and are correctly placed in the Myriangiales. Santesson (**1952**) included the genera of the Cryptotheciaceae in the Arthoniaceae because he found the differences between highly developed species of *Stirtonia* and *Cryptothecia*, in which the asci are aggregated in differentiated fertile areas, and *Arthonia* and *Arthothelium*, in which the asci presumably occur in locules among pseudoparaphyses, too vague to justify the separation of the Cryptotheciaceae. However, pseudoparaphyses are not homologous with inter-

thecial stromal tissue and can not be derived through the compression of such tissue. The differences, therefore, are qualitative rather than quantitative. Developmental studies will be necessary to determine whether these genera form an intergrading series or are properly separated into two families, which are here placed in different orders, the Cryptotheciaceae in the Myriangiales, the Arthoniaceae in the Hysteriales.

Family 5. Stephanothecaceae
Syn. Microthyriellaceae

The concept of the Microthyriellaceae (Luttrell, **1951**) as a family of species with superficial, dimidiate-scutate, inverse ascostromata in which the usually globoid asci are distributed in individual locules apparently corresponds with the vague definition of the Stephanothecaceae by Petrak (**1931**). Consequently, the prior name Stephanothecaceae is used for this family.

Subfamily 1. Protothyrioideae
Subfamily 2. Microthyrielloideae
Subfamily 3. Trichopeltoideae

Order II. DOTHIDEALES
Syn. PSEUDOSPHAERIALES, CAPNODIALES, DOTHIORALES
Family 1. Pseudosphaeriaceae
Syn. Parodiellinaceae, Parodiopsidaceae

The family Pseudosphaeriaceae was erected by von Hoehnel (**1907**) for the genera *Wettsteinina* and *Pseudosphaeria*. *Pseudosphaeria* has since been reduced to synonymy with *Wettsteinina* (Müller, **1950**). The family was characterized by the production of small, innate or erumpent, perithecium-like ascostromata containing a few large asci in individual locules. The name Pseudosphaeriaceae has subsequently been used in an increasingly broader sense for a large group of genera with pseudoparaphysate centra. These pseudoparaphysate forms are entirely different from *Wettsteinina* in structure. It therefore seems advisable to return to von Hoehnel's original definition and limit the family to forms similar to *Wettsteinina*. The family Parodiopsidaceae (Luttrell, **1951**) is essentially similar in conception to the Pseudosphaeriaceae in this restricted sense. Although it contains superficial forms, this family probably should be merged with the Pseudosphaeriaceae. A majority of the genera in Hansford's (**1946**) Parodiellinaceae resemble *Wettsteinina* in internal structure and also may be included in the Pseudosphaeriaceae.

The Pseudosphaeriaceae resemble members of the Dothideaceae in the production of perithecium-like ascostromata with a differentiated, spherical centrum. They differ in that the asci are fewer in number and arise singly, remaining more or less separated by stromal tissue. This structure is clearly shown in Hansford's (**1946**) figure of *Nematostigma obducens* Syd. In this latter respect they resemble the Saccardiaceae. Von Hoehnel (**1907**) considered the Pseudosphaeriaceae similar in internal structure to the Myriangiaceae in that the asci are borne in monascous locules and distinguished the two families by the shape of the stroma. It is evident that the Pseudosphaeriaceae are transitional between the Myriangiales and the Dothideales, some such as *Parodiopsis* and *Parodiellina* resembling the Saccardiaceae more closely, some such as *Wettsteinina* and *Pseudoplea* being hardly distinguishable from members of the Dothideaceae such as *Mycosphaerella*. Consequently, it is of little theoretical importance whether the family is placed in the Myriangiales or Dothideales. For practical reasons, it now seems preferable to include it in the Dothideales, to which it shows a closer superficial resemblance.

At least some of the species, including the type *Pleospora gaeumanni* E. Müller, in Müller's (**1951b**) subgenus *Pseudopleella* of the genus *Pleospora* are more closely related to *Pseudoplea* than to *Pleospora*. These species should be transferred to *Pseudoplea*, or *Pseudopleella* should be raised to generic rank and placed in the Pseudosphaeriaceae as limited here. If this disposition is accepted, Müller's (**1951a**) study of *Pleospora gaeumanni* furnishes the first detailed account of ascocarp development in this family. In *P. gaeumanni* the ascocarp originates from a single hyphal cell which divides in several planes to form a spherical, parenchymatous stroma. A uninucleate ascogonium provided with a trichogyne and a uninucleate antheridium develop within the stroma. Following plasmogamy, the ascogonium produces a short chain of binucleate ascogenous cells. The asci arise directly as side branches from the ascogenous cells. The relatively few, thick-walled asci push up individually into the stromal parenchyma in the center of the ascocarp. At maturity they remain more or less separated by weakly compressed remnants of stromal tissue. The cells in the outer layers of the ascostroma become thick-walled and darkened except at the apex where a lysigenous pore is formed.

Family 2. Dothioraceae

Müller and von Arx's (**1950**) figures of *Dothiora* and other genera of the Dothioraceae indicate that the ascocarp in this family has the

following structure. The ascostroma is pulvinate. It is occupied by a single broad locule filled with a closely packed palisade of aparaphysate asci arising in a single group. Personal observations on a *Leptodothiora* sp. indicate that it is similar in structure. In its development it shows a remarkable resemblance to *Dothidea*, differing only in that the asci instead of arising in discrete groups in several spherical locules arise as a continuous layer in a single broad locule. Strands of tissue between the asci, which are mentioned in some descriptions of species in this family, probably represent remnants of stromal tissue and indicate a connection with the Myriangiales. Since there is no adequate evidence of the occurrence of pseudoparaphyses in the Dothioraceae, this family seems properly placed in the Dothideales rather than in the Pseudosphaeriales. The ordinal name Dothiorales of Müller and von Arx (**1950**), therefore, becomes a synonym of the Dothideales.

Family 3. Dothideaceae

Recent developmental studies on *Systremma* (*Dothidea*) *natans* (Tode) Theiss. & Syd. (Luc, **1952**), *Dothidella* (*Plowrightia*) *insculpta* (Wallr.) Theiss. & Syd. (Hess and Müller, **1951**), and *Mycosphaerella berberides* (Auersw.) Lindau (von Arx, **1949**) are essentially in agreement with previous accounts of development in this family.

Family 4. Capnodiaceae

Order III. TRICHOTHYRIALES

Family 1. Trichothyriaceae

According to Petrak (**1950**), previous accounts of a peculiar inverse development of the ascocarp in this family (see Luttrell, **1951**) are incorrect. The ascocarp originates from a single swollen cell terminating a short branch of the superficial mycelium. This cell produces two plates of cells both of which are more or less radiate in structure. The lower plate forms the base of the ascocarp. It fuses at the margins with the upper plate, which forms the apical wall of the ascocarp. The pore is formed at the apex, while the asci arise from the base and are directed upward toward the pore. The asci arise in a scanty "paraphysoidal" tissue of an indistinctly thready structure which disintegrates at an early stage. While Petrak's account indicates that development in this family is not so unusual as has been supposed, it does not provide a sufficiently clear description of structure and development of the ascocarp to define the correct taxonomic position of this apparently isolated group of fungi.

Order IV. **PLEOSPORALES** nom. nov.

The order Pseudosphaeriales was established by Theissen and Sydow (**1918**), the name being based on the family Pseudosphaeriaceae of von Hoehnel (**1907**), for a series of ascostromatic genera distinguished from the Dothideales by their uniloculate, perithecium-like ascostromata. The production of uni- or pluriloculate ascostromata obviously is not a suitable criterion for separating this group of fungi into orders. Consequently, the Pseudosphaeriales and Dothideales have usually been combined in a single order (Nannfeldt, **1932**; Gäumann, **1949**; Müller and von Arx, **1950**; Munk, **1953**; von Arx and Müller, **1954**) for which the name Pseudosphaeriales has unaccountably been retained in preference to the prior name Dothideales. Miller (**1938**, **1949**) first pointed out that two distinct developmental types occur in this group. In the first (*Dothidea* Type of Luttrell, **1951**) the centrum is composed of pseudoparenchyma which disintegrates as the asci develop. This results in the formation of a locule occupied by a cluster of aparaphysate asci. In the second (*Pleospora* Type of Luttrell, **1951**) the centrum is composed of pseudoparaphyses among which the asci develop later. Miller referred genera of the *Dothidea* type to the Dothideales, those of the *Pleospora* type to the Pseudosphaeriales.

Miller's (**1938**, **1949**) separation of these orders has not been generally accepted because of a false conception of the nature of pseudoparaphyses. Von Hoehnel (**1907**) described the asci of *Wettsteinina* and *Pseudosphaeria* as arising singly in monascous locules in the stroma and at maturity remaining more or less separated by remnants of stromal tissue persisting as interthecial strands. This conception was mistakenly applied to the entire ascostromatic series by Theissen and Sydow (**1918**). According to their interpretation, the asci in all of these forms arise in monascous locules. In some, such as those in the Myriangiales, the interthecial tissue remains between the mature asci. In some it becomes compressed between the developing asci to paraphysis-like strands (pseudoparaphyses [2]), while in others it is ultimately disintegrated completely. Development in all of these fungi, therefore, is fundamentally

[2] The term pseudoparaphyses is used for the distinct, vertical, paraphysis-like hyphae which occupy the locule or perithecial cavity prior to the formation of the asci. They differ from paraphyses in that they ultimately are attached at both ends and, according to the majority of reports, grow downward from the upper portion of the ascocarp. Strands of stromal tissue between the asci are referred to as interthecial tissue. The simpler term paraphysoid might be preferable except for the fact that it has been applied indiscriminately to pseudoparaphyses and interthecial tissue and consequently has become indefinite in meaning.

the same. They differ only in the degree to which the interthecial tissue is crushed, and this character is of little significance in the separation of orders or even genera (Hansford, **1946**, p. 5; Müller and von Arx, **1950**, p. 356). Although this interpretation has been accepted by many subsequent workers (Petrak, **1923**; Nannfeldt, **1932**; Gäumann, **1949**; Bessey, **1950**; Müller and von Arx, **1950**; von Arx and Müller, **1954**), all available evidence indicates that it is entirely false. Pseudoparaphyses do not represent remnants of interthecial stromal tissue but originate as separate paraphysis-like hyphae prior to the formation of the asci. This is indicated by all of the developmental studies that have been made on this group of fungi. In addition to the studies listed previously (Luttrell, **1951**, pp. 37–45), the reports of Urries (**1945**) on *Leptosphaeria cavanillesii* Urries, Holm (**1951**) on *Stigmatea geranii* (Fr. ex Fr.) Fr., and Luttrell (**1953**) on *Glonium stellatum* offer positive confirmation of this interpretation. As Munk (**1953**) stated, "it is evident —and it has been evident all the time—that the filamentous tissue mentioned is a textura porrecta even before the growth of the asci." Since pseudoparaphyses are not homologous with interthecial tissue, the transition even between forms in which the stromal tissue remaining between the asci is compressed to thin strands and those in which pseudoparaphyses are present is not a simple one. The *Dothidea* and *Pleospora* developmental types are distinct and afford an adequate basis for the separation of orders. Miller's (**1938**, **1949**) definition of the Dothideales and Pseudosphaeriales, therefore, is accepted. However, the new name Pleosporales is proposed for the order Pseudosphaeriales as delimited by Miller. The name Pseudosphaeriales has been used in so many different senses that it has become a source of confusion. Furthermore, the type family Pseudosphaeriaceae along with the type genus *Pseudosphaeria* has been transferred to the Dothideales. Pseudosphaeriales, therefore, becomes a synonym of Dothideales. The name Pleosporales is based on the most important family (Pleosporaceae) of the order, and structure in the type genus *Pleospora* has been demonstrated (Cavara and Mollica, **1907**) to be typical of the order as defined by Miller.

Two large families, the Pleosporaceae and Lophiostomataceae, appear to be well established in the Pleosporales. In addition several proposed new families may be placed in this order.

Family 1. Pleosporaceae

The older name Pleosporaceae is used for the family Pseudosphaeriaceae as defined previously (Luttrell, **1951**) after the transfer of *Wettsteinina* (*Pseudosphaeria*) to the Dothideales.

Family 2. Lophiostomataceae
Family 3. Venturiaceae

The family Venturiaceae was proposed by Müller and von Arx (**1950**) for a group of genera, such as *Venturia, Gibbera, Coleroa,* and *Parodiella,* in which the ovoid or ellipsoid, two-celled ascospores are at first hyaline or pale green but usually become olive-brown or grayish-green at maturity. Munk (**1953**) also considered this a natural group. Von Arx (**1952**) provided a further discussion of this family and a key to genera. The inclusion of *Lasiobotrys* is questionable unless pseudoparaphyses can be demonstrated in this genus.

Family 4. Botryosphaeriaceae

Müller and von Arx (**1950**) placed this family of amerosporous genera in the heterogeneous new order Dothiorales. They later (von Arx and Müller, **1954**) gave an extensive treatment of the Botryosphaeriaceae, including a key to genera and revised descriptions of a large number of species. This family, as represented by the type genus *Botryosphaeria* with its perithecium-like locules and pseudoparaphysate centra, seems much less closely related to other families of the Dothiorales, such as the Dothioraceae and Hysteriaceae, than to the Pleosporaceae. It, therefore, is included in the Pleosporales. However, it requires reorganization. Dothideaceous genera such as *Guignardia* and *Auerswaldia* must be excluded since they do not correspond to the *Pleospora* developmental type. *Bagnisiella* appears to be Myriangiaceous in structure and may represent a link between the Myriangiales and Dothioraceae. Although *Myiocopron* and *Ellisiodothis* correspond in centrum and ascospore structure to *Botryosphaeria,* the inclusion of these genera in the same family with *Botryosphaeria* represents undue neglect of stromal characteristics which, although not of primary importance, should not be ignored entirely. These genera, along with those in the family Entopeltaceae of von Arx and Müller (**1954**), are provisionally retained in the Microthyriales.

Family 5. Mesnieraceae

The family Mesnieraceae was set up in the Dothiorales for the single genus *Mesniera* by von Arx and Müller (**1954**). It was distinguished from the Botryosphaeriaceae chiefly by the brown, ridged or warty ascospores and multiple-spored asci. It also seems correctly placed in the Pleosporales.

Family 6. Didymosphaeriaceae

Munk (**1953**) proposed this family for a small group of genera (*Didymosphaeria, Trichothecium, Mullerella,* and *Valsaria*) charac-

terized by two-celled, golden- or olive-brown, often finely punctate ascospores.

Family 7. Herpotrichiellaceae

The Herpotrichiellaceae is a second small family proposed by Munk (**1953**) for *Herpotrichiella, Capronia, Berlesiella,* and the new genera *Didymotrichiella* and *Dictyotrichiella.* Principal characteristics of the family are the hairy ascocarps, the small asci and ascospores, and the greenish-gray color of the ascospores.

Order V. HYSTERIALES

The families included in this order possibly could be placed in the Pleosporales since in both groups the asci develop among pseudoparaphyses. However, the members of the Hysteriales differ in the round or typically elongate, apothecium-like ascostroma which is occupied by a single, broad, often indefinitely limited locule. They probably are more conveniently placed in a separate order. The Hysteriales comprises the Hysteriaceae and possibly three other families made up chiefly of lichen fungi. *Coccodothis* and some genera of the Patellariaceae such as *Tryblidiella* and *Lecanidion* may ultimately find a place in this order.

The Hysteriales corresponds in part to the order Dothiorales of Müller and von Arx (**1950**) which was set up for the families Botryosphaeriaceae, Dothioraceae, Hysteriaceae, and Phacidiaceae. In a later, very extensive treatment of this order they (von Arx and Müller, **1954**) excluded the Phacidiaceae and recognized the families Botryosphaeriaceae, Entopeltaceae, Mesnieraceae, Dothioraceae, Hysteriaceae, and Arthoniaceae. Von Arx and Müller (**1954**) divided the Ascoloculares into three orders, the Myriangiales, Pseudosphaeriales, and Dothiorales. The Pseudosphaeriales and Dothiorales were distinguished as follows: Pseudosphaeriales—asci cylindrical, ovate, or small clavate, usually without a definite stipe; ascospores 1–many-septate; ascocarp opening by a small round pore formed by resorption of an apical papilla and in higher forms often lined with periphysis-like hyphae; "bitunicate Pyrenomycetes." Dothiorales—asci clavate, usually tapering to a stipe at the base; ascospores non-septate to many-septate; ascocarp opening by the rupture of the flat or papillate apex or by a longitudinal split; "bitunicate Discomycetes." The Pseudosphaeriales of von Arx and Müller corresponds to the Dothideales and Pleosporales combined, the difference in treatment resulting from their rejection of the criterion of centrum structure in the separation of these two orders.

The Botryosphaeriaceae seem out of place in the Dothiorales. Although the asci may be considered clavate-stipitate, the locules in *Botryosphaeria* are perithecium-like and the pore is similar to that in genera which von Arx and Müller considered typical Pseudosphaeriales. With the exclusion of the Botryosphaeriaceae, von Arx and Müller's Dothiorales agrees closely with the Hysteriales as treated here, an order of Discomycete-like ascostromatic forms. On the basis of priority, the name Hysteriales is preferable to Dothiorales. The inclusion of the Dothioraceae in this order is debatable and depends on the emphasis placed on centrum structure. Although they appear superficially to be related to the Hysteriaceae, there is no evidence of the presence of pseudoparaphyses which are typical of the Hysteriaceae. Consequently, the Dothioraceae are tentatively placed in the Dothideales, pending developmental studies which will clarify their structure and taxonomic position.

Family 1. Hysteriaceae

Lohman's (**1932**) report of bitunicate asci in *Mytilidion tortile* (Schw.) Sacc., *M. resinicola* Lohm., *Hysterographium minutum* Lohm., and *Gloniopsis brevisaccata* Lohm., and Luttrell's (**1953**) developmental study of *Glonium stellatum* should be noted.

Family 2. Opegraphaceae
Family 3. Arthoniaceae
Family 4. Rocellaceae

The Rocellaceae includes principally fruticose lichens in which the ascocarp appears to be an ascostroma. Santesson (**1949**) has provided an excellent description of its structure in *Dolichocarpus chilensis* R. Sant. In this species the linear ascocarps are even more elongated than in any of the Hysteriaceae, sometimes reaching a length of three centimeters.

Order VI. MICROTHYRIALES
Syn. HEMISPHAERIALES, ASTERINALES.

Centrum structure in the Microthyriales corresponds closely with that in the Pleosporales, and to emphasize this relationship Luttrell (**1951**) placed the members of this order in the Pseudosphaeriales (Pleosporales) as the family Myiocopronaceae. However, the distinctive dimidiate-scutate ascocarp, in which development is basipetal as in the Stephanothecaceae, is probably a sufficient basis for the recognition of a separate order. Although Hemisphaeriales is the first name pro-

posed for this order, the name of the type family Hemisphaeriaceae is not based on that of a genus included in the family. Arnaud (**1925**) segregated the genera with radiate shields in the order Microthyriales. Subsequently the name Microthyriales has often been used for the two orders combined. On the basis of priority Müller and von Arx's (**1950**) name Asterinales must be considered a synonym of the Microthyriales. The Asterinales was composed of unrelated genera assembled on the basis of their asterinoid habit. The Microthyriaceae and the type family Asterinaceae belong in the Microthyriales. The members of the Meliolaceae, Capnodiaceae, and Parodiellinaceae are entirely different morphologically. Most of them are related to the Dothideales.

Family 1. Microthyriaceae
Syn. Polystomellaceae, Stigmateaceae, Asterinaceae.

The separation of the Microthyriaceae, Polystomellaceae, and Stigmateaceae was based on relationship of the mycelium and ascocarp to the substrate. Hansford's (**1946**) Asterinaceae was erected for species producing hyphopodia on the mycelium. All of these distinctions are based on vegetative characters which are of doubtful value in the separation of families.

Family 2. Micropeltaceae
Syn. Hemisphaeriaceae

Subclass II. EUASCOMYCETES
Syn. ASCOHYMENIALES, PLECTASCALES, UNITUNICATAE

In addition to the ascostromatic series in the Loculoascomycetes, there are isolated groups of ascostromatic forms with unitunicate asci which must be retained in the Euascomycetes. The ascostromatic forms in the Euascomycetes apparently have been derived through reduction of the perithecial walls from true Pyrenomycetes. They do not constitute a single related group but apparently have arisen independently at several points. Nevertheless, they are tentatively assembled in two orders, the Coryneliales and Coronophorales. This hypothesis of reduction would be strengthened if the ascostromatic forms could be connected in an intergrading series with the perithecial forms from which they have been derived. Unfortunately, this cannot be done at present. The stromatic Hypocreaceae and Clavicipitaceae exhibit a definite tendency toward reduction of the perithecial walls, but it is extremely doubtful that any relationship could be established between these groups and the members of the Coryneliales and Coronophorales. An alternative

hypothesis that these forms are derived independently of the Pyrenomycetes from the Plectomycetes was advanced for the Coronophoraceae by von Arx and Müller (**1954**) and would apply equally as well to the Coryneliaceae. However, the difficulties involved in this second hypothesis are as great as in the first. There is no adequate evidence for either.

Order I. CORYNELIALES

The two families, Coryneliaceae and Acrospermaceae, included in the Coryneliales resemble each other superficially in the production of elongate, columnar ascocarps but probably are not related. They differ decidedly in ascospore and ascus structure. In the Coryneliaceae the clavate, long-stalked, thin-walled asci disintegrate and free the ascospores within the locule. Personal observations indicate that in *Acrospermum* the tips of the cylindrical asci emerge from the ostiole and the scolecosporous ascospores are discharged forcibly. McCormack (**1936**) furnished adequate evidence that the ascocarp in the Coryneliaceae is an ascostroma. Although Brandriff (**1936**) suggested that the ascocarp of *Acrospermum* is ascostromatic, her study indicated traces of a perithecial wall and true paraphyses in this genus. It is possible that *Acrospermum* should be considered a partially reduced perithecial form and placed in the vicinity of the Clavicipitaceae.

Family 1. Coryneliaceae
Family 2. Acrospermaceae

Order II. CORONOPHORALES

The Coronophorales were previously (Luttrell, **1951**) divided into two families, the Coronophoraceae and Nitschkiaceae. Munk (**1953**) and von Arx and Müller (**1954**) agreed that this separation is not justifiable. Accordingly, Fitzpatrick's (**1923**) Nitschkiaceae is placed in synonymy with the Coronophoraceae.

The only developmental study in the order is that of Luc (**1952**) on *Bertia moriformis*, a species which Munk (**1953**) considered a typical member of the Coronophorales. The ascocarp of *B. moriformis* is an ascostroma consisting of a broad cylindrical base supporting a globular fertile apical portion which contains a single locule. It originates from vegetative hyphae which emerge from the substrate and intertwine to form a hemispherical stroma. These hyphae become compacted into a pseudoparenchymatous tissue. The peripheral cells become thick-walled and darkened. The centrum is composed of thin-walled pseudoparenchymatous cells which disintegrate prior to the formation of the asci,

leaving a cavity filled with mucilaginous material. In the base of the locule is a hemispherical "placenta" of long rectangular cells from the surface of which the ascogenous hyphae arise. The asci are clavate, long-stalked, and thin-walled. The ascus walls break and free the ascospores within the locule where they continue to mature. A plug of thin-walled cells which extends into the outer layers of the stroma at the apex disintegrates and produces a lysigenous pore through which the ascospores are extruded in droplets.

Biciliospora velutina Petr. (Petrak, **1952**), *Scortechinia culicitella* (Berk. & Rav.) Speg., and *Scortechiniella similis* (Bres.) von Arx & E. Müll. (von Arx and Müller, **1954**) appear to be similar in structure, although the ascocarps are not as massive and open by the rupture of a slightly differentiated area in the apex instead of by a pore as in *Bertia moriformis*. The excellent figures of von Arx and Müller indicate that the asci may arise from more than one "placenta" and occur in several distinct clusters within each ascocarp. Their figures also clearly illustrate the "Quellkörper," or rupturing mechanism, which is characteristic of many genera of this order, including the type *Coronophora*. This consists of a group of cells extending downward into the locule from the apex of the ascocarp which, by the absorption of water and swelling, rupture the apex and free the ascospores. The long-stipitate asci extend to various levels in the centrum. Their fasciculate arrangement, however, argues against the inclusion of the Coronophoraceae in the Plectomycetes, where they were placed by von Arx and Müller (**1954**). The long-stipitate, thin-walled asci, which are sometimes thickened at the apex and extend to various levels, and the pseudoparenchymatous centrum suggest a derivation of the Coronophorales from forms related to the Diaporthaceae, but no close connection is evident.

Family 1. Coronophoraceae
Syn. Nitschkiaceae

ERYSIPHALES, TUBERALES, PHACIDIALES.

The inclusion of the Erysiphales in the Ascoloculares (Loculoascomycetes) by Nannfeldt (**1932**), Gäumann (**1949**), and Müller and von Arx (**1950**) is without basis, as was indicated later by von Arx and Müller (**1954**). The asci are unitunicate and the ascocarp is a simple, nonstromatic perithecium. The position of the Tuberales in the Ascoloculares, where they were placed by Müller and von Arx (**1950**), also is untenable since the asci are unitunicate, and von Arx and Müller (**1954**)

have abandoned this position. Although it is uncertain whether the Tuberales should be considered specialized Discomycetes or highly developed Plectomycetes, there is no evidence that they should be considered ascostromatic forms. The Phacidiales are stromatic Discomycetes. The apothecium within the stroma is often reduced to a hymenium of asci and paraphyses and a thin hypothecium indistinguishable from the underlying stroma. Nevertheless, it is doubtful that they can be considered true ascostromatic forms. Since the asci are unitunicate, they cannot be included in the Ascoloculares where they were placed by Terrier (**1942**) and, originally, by Müller and von Arx (**1950**).

PHYLOGENY

The Loculoascomycetes probably arose from forms in the Hemiascomycetes (FIG. 1), although none in the latter group are known at present which give any indication of the origin of the bitunicate ascus. Forms with the bitunicate ascus developed a tendency to produce their ascogonia within a stroma. The ascogenous hyphae penetrated the stroma and formed ovoid asci in individual locules scattered at different levels in the undifferentiated stroma. The species presently known which most closely approach these primordial forms are found among the lower Myriangiales in *Elsinoë* and *Atichia* (FIG. 2). The genus *Myriangium* represents a specialized side branch from the *Elsinoë* type in which the stroma became more massive and differentiated into sterile and fertile regions. The species of *Myriangium* show a progressive development of ascogenous hyphae until in *M. duriaei* Mont. & Berk. the tissue of the fertile region in which the asci are scattered is composed largely of ascogenous cells. Adoption of the lichen habit by forms related to *Elsinoë* or *Atichia* produced the Cryptotheciaceae. The Stephanothecaceae represent a third, asterinoid series from the *Elsinoë* type in which the superficial ascostromata became reduced to a thin layer and developed a shield-like plate of cells over the upper surface.

The main line of development proceeded from *Elsinoë* through the Saccardiaceae, in which the stroma assumed a more definite pulvinate shape and the asci became more elongated and restricted to a single layer. Further development along three closely related lines culminated in forms classified under the Dothideales. In the first, the stroma became spherical with a differentiated central region in which the asci, although closely grouped, remained more or less separated by remnants of stromal tissue. This tendency produced the borderline group Pseudosphaeriaceae. Although the connection is obscure, the origins of the Capnodia-

ceae may be found among the superficial members of the Pseudosphaeriaceae. The Dothioraceae resulted from a second tendency. Here the stroma retained its pulvinate shape. The asci became more numerous and pushed up as a group into a single broad locule. In the third line, the stroma also remained pulvinate, but the asci were restricted to discrete groups in numerous spherical locules as in members of the Dothideaceae. In the Dothideaceae the stroma became differentiated in various ways. In some such as *Scirrhia* spp. the locules were formed in perithecium-like protuberances from the basal stroma. Simple Dothideaceae such as *Mycosphaerella* could be derived as easily from forms such as *Scirrhia* by reduction of the basal stroma as from those in the Pseudosphaeriaceae. Possibly the origins of the simple, perithecium-like forms should be sought in both of these lines.

Another distinct line of development from fungi in the Saccardiaceae produced the groups characterized by a pseudoparaphysate centrum, the Hysteriales, Pleosporales, and Microthyriales. Those closest to the primitive forms in these groups are probably found in the Hysteriales. Here the stroma is pulvinate or in higher forms elongate-linear. The asci are crowded in a continuous layer in a single broad locule. The locule is formed in part by the disintegration of stromal cells as in the Dothideales, but a second type of locule formation appears. Stromal cells above the ascogenous hyphae produce hyphal outgrowths (pseudoparaphyses) which grow downward and by mechanical pressure assist in the formation of the locule. In higher forms in the Pleosporales the asci are restricted to spherical locules. The pseudoparaphyses become more numerous, and the locule is created almost entirely by their growth. The locule, therefore, is schizogenous in origin rather than lysigenous as in the Dothideales. The stroma in the Pleosporales shows the same variations as in the Dothideaceae. The locules may develop in perithecium-like projections seated on a basal stroma or subiculum as in *Rosenscheldia* and *Curcurbitaria*. Reduction of the basal stroma would produce uniloculate forms such as *Leptosphaeria* and *Pleospora*. The Microthyriales are superficial forms in which the stroma is flattened and covered by a shield-like layer as in the Stephanothecaceae. Usually the locules are similar in structure to those of the Pleosporales. There are several genera, such as *Polystomella*, with elongate, indefinitely limited locules resembling those of the Hysteriales. These point to a derivation of the Microthyriales from pseudoparaphysate forms more primitive than those now included in the Pleosporales, the order to which the Microthyriales appear to be most closely related.

DISCUSSION

The system presented here represents primarily an attempt to classify morphological data. It is based on the developmental studies that have been made in the groups of fungi under consideration. Such studies, including even the most superficial, have been made on only an insignificant percentage of the total number of species. Furthermore, few of these species have been reinvestigated, and the original accounts may not prove to be entirely correct. The separation of developmental types is much like the separation of species. At first, when few studies are available, developmental patterns seem to be clearly distinct. Further study will undoubtedly reveal variations and intergradations to such an extent that the lines will not appear so sharply drawn, and application of the system can be expected to become more difficult. Taxonomists who have approached the problem through the relatively superficial comparison of long series of fungi rather than detailed observation of a few are already better acquainted with variations and may be in a better position to determine how well such a system applies in practice.

Any revision based on the relatively few developmental studies that have been made can be considered only as a tentative hypothesis. On the other hand, there is no need to disparage the considerable body of morphological data that has accumulated or to ignore it in classification. The system of Nannfeldt (**1932**), which finds a logical extension in the erection of the subclass Loculoascomycetes, appears to be supported by present data. The line of thought, well represented by Petrak (**1923**), and Müller and von Arx (Müller and von Arx, **1950**; von Arx and Müller, **1954**), which attempts to demonstrate numerous connections and transitions between the ascostromatic and perithecial forms may ultimately prove to be correct. Nevertheless, it should be understood that this system is founded on a misconception of the nature of interthecial stromal tissues, pseudoparaphyses, and paraphyses which has persisted only because the not inconsequential number of developmental studies on these fungi have been completely disregarded. It is evident that morphological data must be fully utilized if a satisfactory system of classification is to be developed.

GEORGIA EXPERIMENT STATION,
EXPERIMENT, GEORGIA

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OBSERVATIONS ON GYMNOASCACEAE. I. MYXOTRICHUM UNCINATUM AND A NEW SPECIES OF MYXOTRICHUM [1, 2]

HAROLD H. KUEHN

(WITH 36 FIGURES)

Taxonomy within the Gymnoascaceae, even at the generic level, is often not well established and, therefore, identification of species may be difficult or uncertain. A study of the original literature, as well as of Saccardo's treatise of members of this family in the Sylloge Fungorum, shows clearly that the diagnoses and descriptions are often too inadequate to permit conclusive identification of many of the Gymnoascaceae which might be isolated by an investigator. At the present time a satisfactory monograph of the Gymnoascaceae would be difficult to prepare due to a lack of detailed information concerning the structure and development of these fungi. It is difficult, when they reach maturity, to distinguish among species in some of the genera on the basis of ascocarps alone. Therefore, isolates should be studied in culture so that the cultural and developmental characteristics may be utilized also. Little work on the developmental morphology of the Gymnoascaceae has been reported while none of the developmental characters has been applied as an aid in the identification of species.

The development of many species of the Gymnoascaceae has been examined by the author to determine whether a sounder basis for taxonomy might be established through the additional application of developmental morphological characters. Observations made on two species of *Myxotrichum* are reported in this paper. Additional papers will pre-

[1] This paper represents a portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany at the University of Illinois, August, 1954.

[2] The writer wishes to express his sincere appreciation to Dr. Leland Shanor under whose supervision this investigation was accomplished. Grateful acknowledgment is made to Dr. C. W. Emmons and Dr. R. K. Benjamin for supplying cultures of many of the fungi examined, and to Dr. C. W. Hesseltine for making available the Gymnoascaceae in the culture collection of the Northern Utilization Research Branch of the USDA.

sent the results obtained from the study of other species of *Myxotrichum* and species belonging to other genera.

HISTORICAL

Kunze and Schmidt (**1823**), in describing *Myxotrichum chartarum* and *M. murorum*, provided descriptions and diagnostic figures of the first members of fungi now included in the family Gymnoascaceae. Eidam (**1880**) described a new species, *Gymnoascus uncinatus*, and reported on the development of the cleistothecium. This species was transferred to *Myxotrichum* by Schroeter (**1893**). According to Eidam, the gametangia in this species arose either from two different hyphae or from the same hypha and spiralled about each other. One gametangium, the antheridium, remained short, became swollen at the apex, and eventually a cell was delimited terminally by a septum. The ascogonium coiled about the antheridium and became septate, after which the lower cells put forth rhizoids while the upper cells branched repeatedly to produce a thick tuft of short branches each of which became swollen at its apex to form an ascus. Branches of the vegetative hyphae formed a covering around several pairs of gametangia. As the asci matured these vegetative hyphae became yellow and thick-walled (cuticularized) to form a loose peridium, the hyphae of which ended partly in short blunt tips and partly in longer appendages which were inrolled at the apex. Eidam stated that no mature ascocarps were formed on artificial media. Instead, development ceased after the spiral ascogonium became septate and produced branches.

Eidam reported that intercalary or terminal swelling of aerial condiophores resulted in conidia which separated at maturity. These spores were reported as variable in shape, being round, oval, lemon-shaped, or rounded at the ends.

Nannizzi (**1926**), the only investigator since Eidam to report a study of the morphology of *M. uncinatum*, depicted a completely different pattern of development. He reported that no antheridium was formed. From certain hyphae he observed that a very short lateral branch developed, and, after it was cut off by a septum, this enlarged immediately to form a swollen cell. This swollen cell functioned as an ascogonium, and gave rise to several ascogenous hyphae which branched repeatedly with asci forming at the apices. Nannizzi stated that the cleistothecia of his fungus differed from those typical of *M. uncinatum* in the possession of fewer and longer appendages. Benjamin [3] in 1951 obtained

[3] Unpublished notes.

in pure culture an isolate of *Myxotrichum* which he found was similar, both in development and in its mature structure, to that described by Nannizzi. Benjamin was of the opinion that Nannizzi did not study *M. uncinatum*, but rather an isolate representing a new species.

There have been many papers of a strictly taxonomic nature published describing new species of *Myxotrichum*. However, other than the papers of Eidam and Nannizzi, there have been no reports of morphological studies of species of *Myxotrichum*.

Studies on morphological development of other species of Gymnoascaceae have been reported by Dale (**1903**), DeLamater (**1937a**, **1937b**), Rosenbaum (**1944**), Hotson (**1936**), Dangeard (**1907**), Matruchot and Dassonville (**1901**), Baranetzky (**1872**), Eidam (**1880**, **1882**), Brefeld (**1891**), Van Tieghem (**1887**), Saccas (**1950**), and Emmons (**1935**).

MATERIALS AND METHODS

A variety of media suffice for the growth of the Gymnoascaceae. In this investigation the fungi were grown on Sabouraud's agar, with either maltose or dextrose as the sugar source, Czapek's solution agar, PDA, and Yp. Ss. agar. The latter medium, devised by Emerson (**1941**) particularly for the cultivation of *Allomyces*, proved excellent also for growth of Gymnoascaceae. Since the development of diagnostic characteristics in certain species seems to be dependent upon particular substrata, the appearance of each species growing on the various media is described. The fungi were cultured in Petri dishes and examined frequently in order to ascertain when the inception of the sexual phase occurred. The preparation of permanent mounts of suitable material for examination with the microscope is an operation which requires considerable patience. Minute tufts of hyphae were removed with extremely fine watchmakers forceps, immersed for several seconds in 95% ethanol in order to remove air, and placed in a drop of lactophenol on the slide. During this operation extreme care was exercised in order to prevent the hyphae from becoming entangled or intertwined. If this happened their original orientation was destroyed and the gametangia were obscured. Olive (**1944**) pointed out that often crozier formation in Ascomycetes has not been detected because the only material studied had been sectioned. He described a technique for preparing slides for the purpose of detecting croziers. A modification of his procedure has been used in this study with excellent success. The ascocarps, both mature and immature, were placed on a slide, first in a drop of 95% ethanol to remove air, and then in a drop of lactophenol with acid fuchsin.

The peridium of the cleistothecium, while being observed with a dissecting microscope, was carefully removed from around the central clump of ascogenous hyphae. Then the cover slip was put in place and the slide was transferred to the stage of a compound microscope for further study. The immature asci and croziers were spread out on the slide by pressure exerted on the cover slip with a fine dissecting needle. The effect of this pressure was followed by continuous observation through the microscope. When the material had been macerated satisfactorily, the edge of the cover slip was ringed with finger-nail polish to prevent excessive drying out of the preparation.

OBSERVATIONS

MYXOTRICHUM UNCINATUM (Eidam) Schroeter
Gymnoascus uncinatus Eidam

The diagnoses of *Myxotrichum uncinatum* presented by Saccardo (**1889, 1906**), Eidam (**1880**), Schroeter (**1893**) and Massee and Salmon (**1901**) are either incomplete in certain respects or differ from one another in some details. Therefore, it is desirable at this time to give a more complete description, including cultural characteristics. The isolate of *Myxotrichum uncinatum* which was selected, from the standpoint of characteristics of mature cleistothecia, fits the description of this species as presented originally by Eidam.

On Yp. Ss. agar the colonies possess two types of mycelial areas. One type is cottony or flocculent and white, while the other type is cream colored, restricted, and consists of conidial structures. Cleistothecia appear in about 23 days as white tufts of hyphae in the cottony areas. The reverse of the cottony areas is yellow, with the pigment diffusing into the medium for a short distance; the reverse of the conidial areas is dark-brown.

On Sabouraud's agar the colony characteristics are essentially the same as on Yp. Ss. agar, with the exception that with age the colonies become green, brown, or yellow-brown in different areas. Also, cleistothecia do not appear so soon, and often do not develop for two to three months.

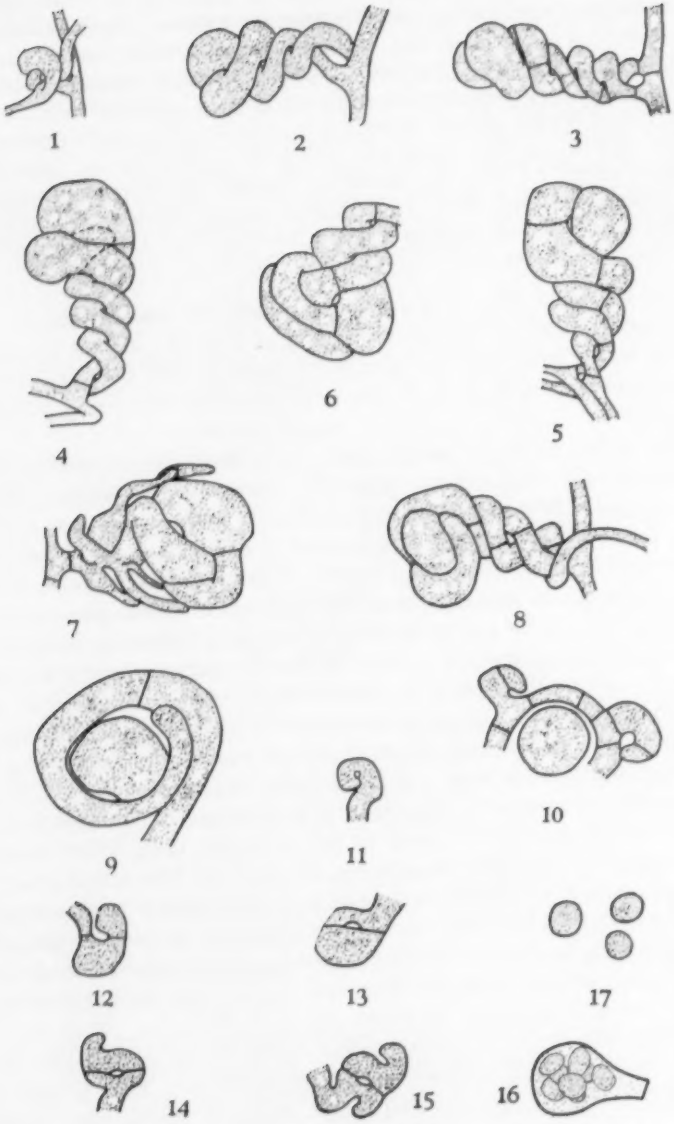
Cleistothecia spherical, red-brown, yellowish or orange-brown in culture, light yellow-brown as seen under the microscope, 429–795 μ diameter, including the appendages. Peridium composed of yellow-brown, cuticularized, asperulate, septate hyphae with appendages which are partly short, blunt spines but mostly are longer and with uncinate apices. Both types of appendages may be branched one to several times.

The short asperulate appendages are 2.5–3.4 × 5.2–16.7 μ. The longer uncinate appendages are light brown, asperulate toward the base, smooth otherwise, 5.1–5.8 × 75–353 μ. If such an appendage branches, the branches are also uncinate terminally. Asci hyaline, obovate to spherical, 6.7–7.2 × 8.4–9.8 μ, and contain eight ascospores. The ascus walls are ephemeral, with the spores remaining in a ball. Ascospores hyaline to slightly yellow, smooth, spherical, 2.8–4.2 μ in diameter. Vegetative hyphae hyaline, 1.4–5.6 μ in diameter. Racquet mycelium also present. The imperfect phase consists of asperulate conidia borne singly on short straight conidiophores; conidia obovate, 3.8–4.2 × 4.2–5.6 μ, or spherical, 8.1–8.6 μ in diameter.

The isolate of this species which was studied was present in the mycological culture collection, University of Illinois. It had been isolated by R. K. Benjamin from goat dung, March, 1949, Urbana, Illinois. This isolate is designated as Benjamin's culture *56* and also as NRRL [4] A–*3610*.

This isolate possesses gametangial initials which are morphologically similar from the start and are quite different from what Eidam (**1880**) had reported. From the same hypha (FIG. 3) or from different hyphae (FIG. 1), two branches arise and proceed to coil about one another two or three times in a symmetrical spiral. The initials are slender at the base, increasing in diameter to an enlarged apex. Each gametangium is separated from its parent hypha by a septum, following which each becomes several-septate (FIG. 3). Fusion of the gametangia was not detected. From the enlarged apical cell of one of the initials, the ascogonium, a slender prolongation grows out (FIG. 4), continues to coil about the other branch, the antheridium, and becomes separated by a septum from the branch which gave rise to it (FIGS. 5–7). Septa divide the prolongation into several ascogenous cells. Ascogenous hyphae are produced from many of the ascogonial cells. They remain short and form croziers immediately (FIG. 10). The initial croziers do not form asci, but instead the penultimate cell produces another crozier (FIGS. 14, 15). Ultimately, asci are formed from the penultimate cells. Approximately at the time when the prolongation is produced from the ascogonium, slender vegetative branches appear from the lower cells of the gametangia and grow up about the coil (FIG. 7). They do not grow down into the substratum to serve as rhizoids, as Eidam described, nor do they take part in formation of the peridium. They branch repeatedly to form a type of filler tissue about the ascogenous hyphae

[4] Northern Regional Research Laboratory, USDA, culture collection designation.



FIGS. 1–17.

within the ascocarp. Vegetative hyphae surrounding one to several pairs of gametangia become differentiated to form the peridium. Although many of the cleistothecia are simple, containing asci having their origin from only one pair of gametangia, compound ascocarps, derived from more than one pair of gametangia, also are formed.

The isolate of *M. uncinatum* examined in this investigation is quite different in its developmental morphology from that described previously for this species by Eidam (**1880**) and by Nannizzi (**1926**). As has been pointed out already, Nannizzi, in all probability, was working with a species other than *M. uncinatum*. It is impossible at this time to explain the difference in gametangial relationships as reported by Eidam and those observed during the course of this investigation. Results of morphological investigations of other species of Gymnoascaceae by the author (**1954**) indicate that different isolates of the same species have the same type of gametangial morphology.

Myxotrichum emmonsii sp. nov.[5]

Cleistotheciis globosis, 189–418 μ diam. appendiculis exclusis, pallide vel atro-brunneo. Peridii hyphis asperulatis, septatis, 2.6–3.0 μ diam. Appendiculis biformibus, vel brevibus, asperulatis spinis 7.0–42.0 μ longis, vel longis, aseptatis appendiculis, interdum laxe vel haud perfecte uncinatis ad apicem, sed saepe rectis, omnibus levibus, ad basim asperulatis, 2.8–4.2 $\times$ 302–644 μ. Ascis hyalinis, ovatis vel subglobosis, 5.2–5.6 $\times$ 6.5–7.0 μ diam., octosporis. Ascosporis hyalinis, globosis vel leviter ovoideis, echinulatis, globosis 2.1–2.8 μ diam, ovoideis 2.1–2.6 $\times$ 2.8–3.2 μ. Hyphis sterilibus flavo-luteis. Oidiis hyalinis, 1.4 $\times$ 2.6–7.0 μ.

Cleistothecia spherical, 189–418 μ in diameter, exclusive of the appendages, light to dark brown in culture, yellow-brown as seen under

[5] The writer acknowledges with appreciation the assistance of Dr. D. P. Rogers, New York Botanical Garden, in the preparation of the Latin diagnosis.

FIGS. 1–17. *Myxotrichium uncinatum*. 1. Very young gametangia arising from different parent hyphae. 2. Coil showing extent to which mutual coiling occurs prior to septation. 3. Gametangia arising from the same parent hypha, with one initial already septate. The apex of each initial has become enlarged. 4. A prolongation arises from the swollen apical cell of the ascogonium. 5. The prolongation has become separated from the ascogonium by a septum. The gametangia have arisen from separate hyphae. 6. The prolongation has coiled two times about the enlarged terminal cell of the antheridium. 7. Thin vegetative hyphae arising from the base of the coil. The gametangia are from the same parent hypha. 8. The coiling initials, each of which are septate several times. 9. A young coil as seen from above. 10. A coil as seen from above showing ascogenous hyphae with croziers arising from the septate ascogonium. 11–13. Stages in crozier formation. 14–15. Stages in development of the penultimate cell to form another crozier. 16. Mature ascus. 17. Mature ascospores. All figures $\times$ 1300.

a microscope. The peridium is composed of light yellow, asperulate, septate, cuticularized hyphae, 2.6–3.0 μ in diameter. The appendages are of two types: short, asperulate spines 7.0–42.0 μ long; and long, non-septate appendages, sometimes loosely or incompletely hooked at the apex but often straight, smooth except near the base where they are asperulate, 2.8–4.2 × 302–644 μ. Asci hyaline, oval to subspherical, 5.2–5.6 × 6.5–7.0 μ, and contain 8 ascospores. Ascus wall ephemeral, with the spores adhering in a ball 6.6–8.4 μ in diameter. The ascospores hyaline, spherical to slightly ovoid, pronouncedly echinulate; spherical spores measure 2.1–2.8 μ in diameter, while ovoid spores have dimensions of 2.1–2.6 × 2.8–3.2 μ. Vegetative hyphae yellow-orange, 1.2–1.5 μ in diameter, not including the racquet mycelium which is present also. The imperfect spore phase is represented by hyaline oidia which measure 1.4 × 2.6–7.0 μ.

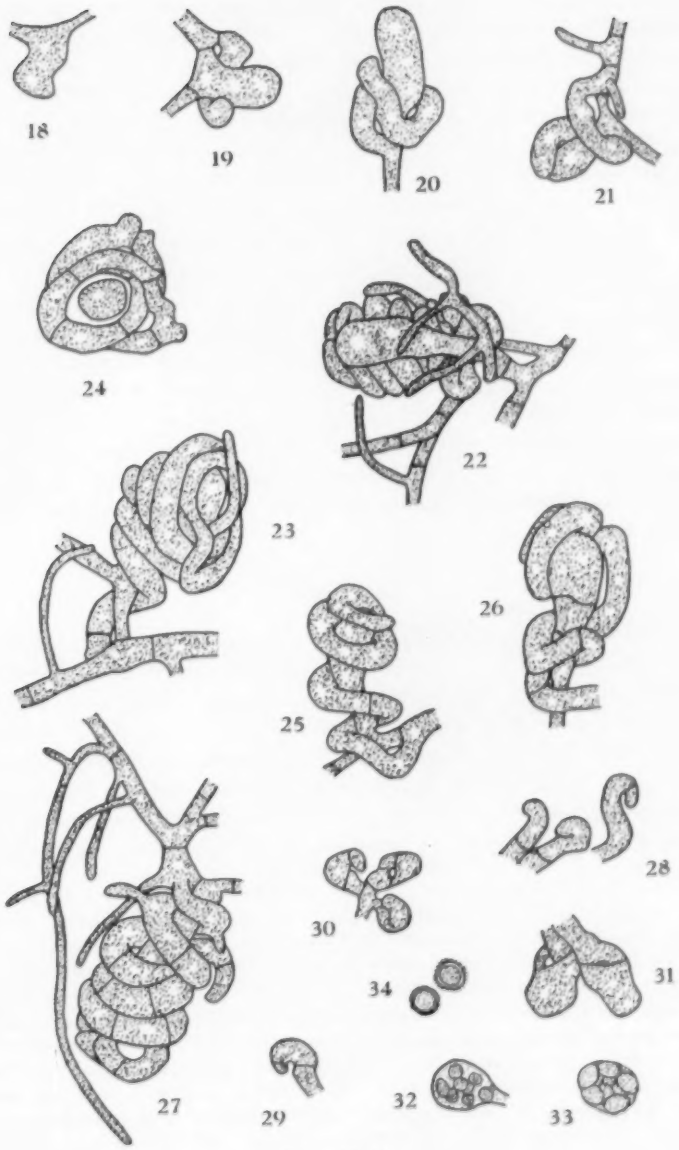
Two isolates representing this species, designated as *4950* and *4951*, were provided by Dr. C. W. Emmons. They were isolated from bat dung in Georgia. This species is easily distinguished from *Myxotrichum uncinatum* because it forms relatively few appendages per ascocarp. These appendages are long and often straight. If hooked terminally, they are not so conspicuously uncinate as in *M. uncinatum*. *Myxotrichum emmonsii* is separated from other species of *Myxotrichum* in having light or dark brown ascocarps, echinulate ascospores, and yellow-orange vegetative hyphae.

Colonies upon Sabouraud's maltose agar are deep and flocculent, at first white, but later becoming yellow-orange, with the intensity of pigmentation varying from place to place. Reverse of the colony is red-orange from the start, with the pigment not diffusing into the medium. Ascocarps appear in 2–3 weeks as white tufts of hyphae and mature within one week. Mature cleistothecia are brown and are either scattered upon the surface of the colony or aggregated into dense clusters up to 5 mm deep and extending throughout the colony.

Colonies on Sabouraud's dextrose agar are slow-growing, often not covering the surface by the time the agar has dried down. The white, flocculent mycelium becomes slightly orange or tawny. Ascocarps appear as they do on Sabouraud's maltose agar, but are not so numerous. The reverse of the colony appears as it does on Sabouraud's maltose agar.

Colonies on Czapek's agar are slow-growing, restricted, otherwise as on Sabouraud's dextrose agar. Cleistothecia are more numerous than on Sabouraud's dextrose agar.

The gametangial initials arise from the same or from different hyphae, with the antheridium forming first as a straight, club-shaped



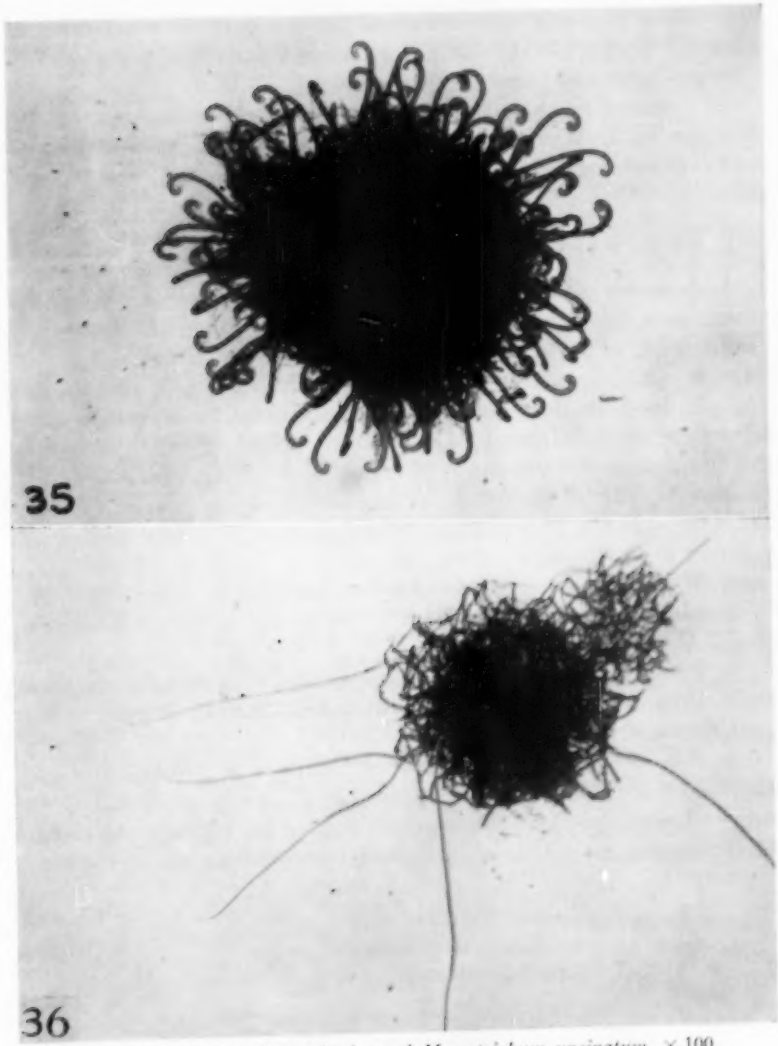
FIGS. 18–34.

structure (FIG. 18). The slender ascogonium arises near the base of the antheridium (FIG. 19), following which each gametangium becomes delimited from its parent hypha by a septum. After the antheridium becomes once or twice septate (FIG. 26) it ceases to elongate, while the ascogonium coils about it 3 to 5 times in a tight (FIG. 23) or loose (FIG. 25) spiral. Fusion of the gametangia could not be detected. Slender vegetative branches arise from the base of the coil or from the parent hypha below the gametangia (FIGS. 21, 22, 27). The vegetative branches grow up around the coil, but do not take part in formation of the peridium. Septa form in the ascogonium, dividing it into several cells, many of which produce ascogenous hyphae (FIGS. 24, 27). Typical croziers are formed, but the penultimate cell grows out to form additional croziers. The cell formed by fusion of the ultimate and antepenultimate cells also produces an ascogenous hypha which recurves to form a crozier. A cluster of ascogenous hyphae is formed in this manner with the eventual penultimate cells of croziers ultimately becoming asci (FIGS. 30, 31). Vegetative hyphae in the vicinity of the coil become differentiated to form the peridium. Compound cleistothecia are formed frequently.

DISCUSSION

The presence of croziers in these two species of *Myxotrichum* brings to six the total number of species of Gymnoascaceae which have been reported to possess such structures. Emmons (**1935**) also reported the formation of croziers in *Byssochlamys fulva* Olliver & Smith, a species which has been regarded by most investigators (see Ainsworth and Bisby, **1950**) as belonging to the Endomycetales. However, it would seem that the genus *Byssochlamys* should more properly be classified among the Gymnoascaceae in the Aspergillales (Eurotiales).

FIGS. 18–34. *Myxotrichum emmonsii*. 18. Young antheridium arising as a short lateral branch. 19. Gametangia arising on the same parent hypha, with the ascogonium starting to coil about the antheridium. 20. An ascogonium arising from the lateral branch which bears the antheridium. 21. Vegetative branches growing out from the parent hypha below the coil. 22. An optical section of the coil, with the ascogonium coiled tightly about the antheridium. Slender branched vegetative hyphae arise from the base of the coil. 23. Advanced stage of coiling, with the initials non-septate. 24. Coil as seen from above, with ascogenous hyphae arising from the septate ascogonium. 25. A rather loose coil, with one septum in the ascogonium. 26. An ascogonium and antheridium, each twice septate. 27. A septate ascogonium with young ascogenous hyphae. 28–31. Stages in crozier and ascus formation. 32. A mature ascus. 33. A group of ascospores following disappearance of the ascus wall. 34. Mature ascospores. All figures × 1300.



FIGS. 35, 36. 35. Cleistothecium of *Myxotrichum uncinatum*, × 100.
36. Cleistothecium of *Myxotrichum emmonsii*, × 100.

DeLamater (**1937a**) was the first to illustrate croziers among generally recognized members of the family, reporting them for a species of *Arachniotus*. The same year he also described (**1937b**) croziers of *Eidamella spinosa* Mat. & Das. Rosenbaum (**1944**) showed that

Arachniotus trisporus Hotson formed asci through the intervention of croziers. Kuehn (**1954**) investigated representatives of six genera of Gymnoascaceae and found croziers present in all of the species studied.

In a second paper it is intended to present observations on certain other species of *Myxotrichum*. A discussion of comparative developmental morphology of species of the genus is being reserved for that paper.

SUMMARY

1. A search of the literature on the Gymnoascaceae has clearly revealed that the descriptions and diagnoses are often inadequate for identification of these fungi. Therefore, an investigation was undertaken to establish sounder bases for classification of the Gymnoascaceae.

2. In an effort to determine whether gametangial morphology might serve as an additional taxonomic character for the separation of species, the morphological development of many species of Gymnoascaceae was examined. The observations on *Myxotrichum uncinatum* (Eidam) Schroeter and for *M. emmonsii*, described here as a new species, are reported in this paper.

3. *Myxotrichum uncinatum* possesses gametangia which are similar in appearance in the early stages. This is not in accord with what Eidam has reported.

4. The gametangia of *M. emmonsii* consist of a central club-shaped antheridium which becomes encircled several times by a more slender ascogonium.

5. In both species croziers are formed prior to formation of asci. Indications are that croziers are formed in all species of the family and may be looked upon as a characteristic of some phylogenetic significance in establishing the relationship of the Gymnoascaceae among the lower Ascomycetes.

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ADDITIONS TO THE PHYCOMYCETE FLORA OF THE DOUGLAS LAKE REGION. I. NEW TAXA AND RECORDS

F. K. Sparrow and Margaret E. Barr[1]

(with 27 figures)

In the course of a continuing investigation of the phycomycetous flora of the Douglas Lake region in Northern Michigan (Sparrow, **1952**) several interesting new forms were found in 1954 which it is the purpose of this paper to record. One, *Chytridium mucronatum*, which was recovered only from Chippewa County in the Upper Peninsula, is also included, as are new records of already described species.

A new variety of Micromyces ovalis

Rieth (**1950**) has recently described from Austria a member of the Synchytriaceae, *Micromyces ovalis*, with strikingly ornamented prosori. These were covered in seriate fashion with coarse "shark-tooth like" spines, wholly unlike the ornamentation of any other species of the genus. We have found in *Zygnema sp.* a precisely similar form (Fig. 21). In contrast to Rieth's material, however, ours was constantly twice the size, being 36–40 × 16–21 μ, as compared with 12–18 × 8 μ. We consider this form sufficiently distinctive to segregate as a variety of *M. ovalis*.

Micromyces ovalis Rieth var. **giganteus** var. nov.[2] Fig. 21

Prosorus hyalinus vel luteolus, ellipsoidalis, 36–40 μ longus, 16–21 μ diam., lineas sex circulares spinularum ferens, spinulis 4–6 μ longis. Sporangia et zoosporae ignotae.

Parasiticus in cellulis deformatis *Zygnematis* immodice elongatis ad locum palustrem "Smith's Bog" dictum, Cheboygan Co., Mich., 24 Jun. 1954.

Prosorus hyaline to light yellow, ellipsoid, 36–40 μ long by 16–21 μ in diameter, bearing six circular rows of spines, 4–6 μ long; sorus, sporangia and zoospores not observed.

[1] Contribution No. 1030 from the Department of Botany, University of Michigan, and from the University of Michigan Biological Station.

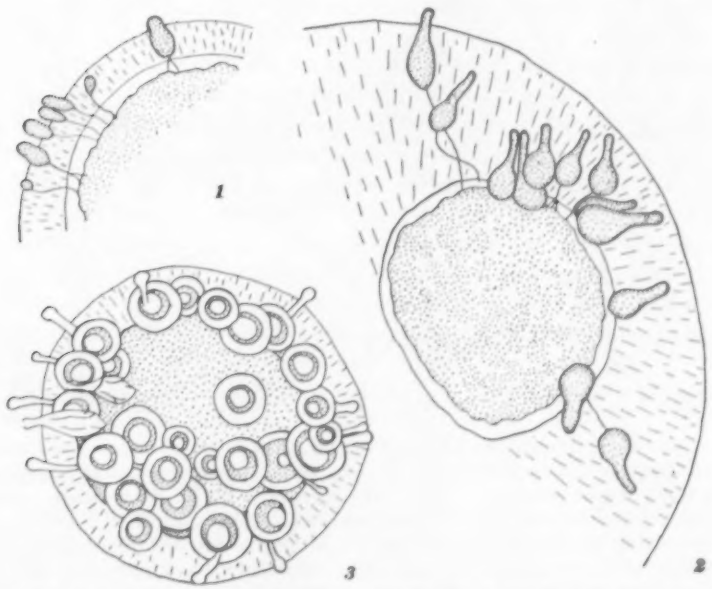
[2] We are greatly indebted to Prof. H. H. Bartlett for preparing the Latin descriptions of new taxa.

Parasitic in and causing elongation of the cells of *Zygnema sp.*, Smith's Bog, Cheboygan Co., Michigan, June 24, 1954.

A NEW SPECIES OF DANGEARDIA

A second most interesting form belonging to the genus *Dangeardia* was observed in limited numbers on an alga tentatively identified by Gilbert M. Smith as *Glocodinium sp.* Its development is as follows.

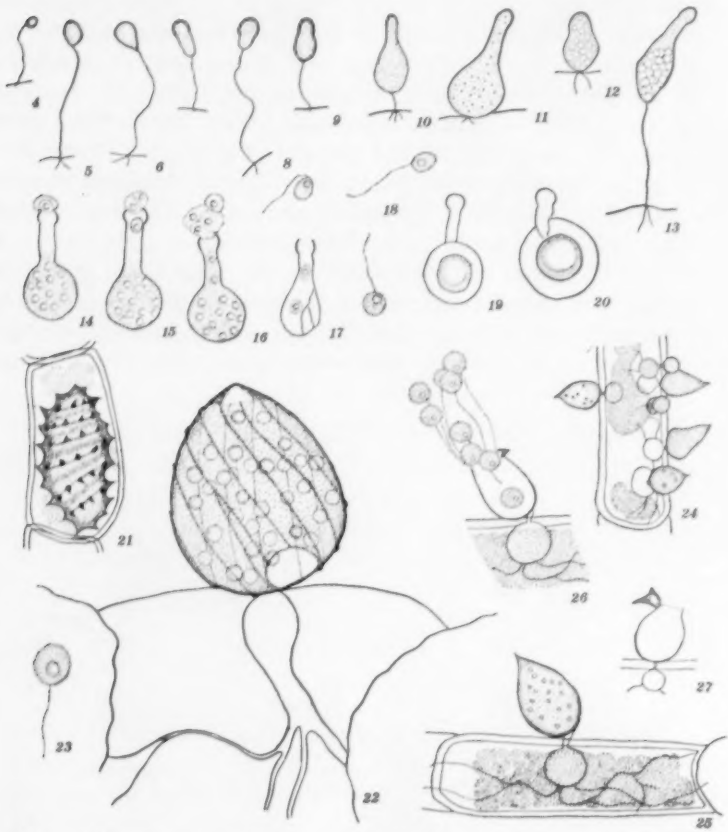
The zoospore comes to rest, encysts on the surface of the thick gelatinous sheath of the host, and produces a long slender tube which penetrates to the dense mass of host material (FIGS. 1, 4). There then arise from the tip of this tube two or three short, unbranched rhizoids which penetrate the host cell contents (FIGS. 5, 6). The long penetration tube enlarges below the body of the encysted zoospore until it is nearly as broad as the cyst (FIGS. 7, 8). The most distal portion of this enlargement expands still further to form the globose portion of the sporangium, whose base generally rests on the host cell contents (FIGS. 2, 9–11). The rhizoids arise either directly from the sporangial



FIGS. 1–3. *Dangeardia laevis* on *? Glocodinium sp.* 1. Young sporangia developing on the host. 2. Older sporangia. 3. Resting spores and two empty, collapsed sporangia seated on the host and immersed in the gelatinous sheath, × 370.

base or from a very short basal stalk. The upper portion of the sporangial rudiment remains unchanged in width and forms the neck of the sporangium which is terminated by the thicker walled conspicuous zoospore cyst.

The contents of the immature sporangium are at first finely granular



FIGS. 4–20. *Dangeardia laevis.* 4–13. Stages in the development of sporangia. 14–16. Steps in the discharge of zoospores. 17. Collapsed sporangium containing two undischarged zoospores. 18. Three zoospores. 19, 20. Two resting spores, × 490. FIG. 21. *Micromyces ovalis* var. *giganteus,* prosorus in cell of *Zygnema sp.,* × 490. FIGS. 22, 23. *Blyttiomyces helicus* on pine pollen. 22. Sporangium. 23. Zoospore, × 1230. FIGS. 24–27. *Chytridium mucronatum* on *Oedogonium sp.* 24. Thalli in different stages of development. 25. Mature sporangium just prior to zoospore discharge. 26. The same sporangium at time of zoospore discharge, zoospores becoming freed from the cluster. 27. Empty sporangium with attached operculum, × 615.

but later become shot through with numerous refractive, colorless globules (FIGS. 12, 13). After the zoospores are delimited in the usual chytridiaceous fashion, they become clustered in the globose basal portion of the sporangium. At zoospore discharge the distal part of the thick-walled neck deliquesces and the first emerging zoopores ooze slowly out in close succession and become clustered at the orifice (FIGS. 14–16). Here they remain quiescent for a very short time, then separate and dart off (FIG. 18). Laggard zoospores emerge individually and immediately swim away. Subsequent to zoospore discharge the sporangium collapses (FIG. 17).

The resting spores occupy the same position on the host as do the zoosporangia, *i.e.* on the surface of the contents proper (FIG. 3). The zoospore cyst on the surface of the sheath and the long, broad empty germ tube are persistent. The resting-spore wall is thick and smooth and encloses the finely granular contents within which is a large, eccentric, refractive globule (FIGS. 19, 20). No paired zoospores were observed, such as described by Canter (**1946**) as preceding resting spore formation in *Dangeardia mammillata*, so it must be assumed, unless complete fusion of planogametes has occurred out in the medium and has been overlooked, that the resting spores are asexually formed.

A comparison of our *Dangeardia* with the only other described species of the genus, *D. mammillata* Schröder, indicates a striking similarity in the method of development, zoospore discharge, etc. of the two. The resting spores of the two species, however, while similar in position with respect to the host, differ markedly in two important features. Firstly, in *D. mammillata* they are formed after a conjugation of immature thalli whereas in the new species there is no evidence for a sexual process. Secondly, in Schröder's species the wall of the resting spore is spiny whereas in our material it is smooth. Other differences between the two might be pointed out, such as the generally larger sizes of the parts of the Michigan fungus, host plants, etc.

For these reasons we consider our fungus distinct from *Dangeardia mammillata*.

Dangeardia laevis sp. nov. FIGS. 1–20.

Zoosporangium pyriforme in vagina gelatinosa immersum, 22–38 μ longum, 8–16 μ diam., parte angustata basali 4.8 μ diam., parte superiore expansa zoosporas includente 8–16 μ diam., membrana laevi, hyalina, crassiuscula; rhizoideis pluribus, brevibus, simplicibus; zoosporis globosis vel ovoideis, aliis primum emergentibus nondum motitantibus ad orem aggregatis sed mox natantibus, aliis singulatim emergentibus cito saltantibus. Sporae durantes in vagina gelatinosa hospitis probabiliter *Gloeodinii* globosae, tubo longo, lato, persistenti praeditae, a vesica zoo-

sporarum aliquantulum latiore et crassiore terminato. Vesica 13–21 μ diam., ut videtur non sexualiter producta, intus granulosa, 8–15 μ diam., globulo eccentrico magno praedita; membrana 2.5–3 μ crassa, laevi, hyalina. Germinatio nondum observata.

Parasitica in alga gelatinosa probabiliter *Gloeodineo*, ad locum palustrem "Smith's Bog" dictum, Cheboygan Co., Mich., 3 Jul. 1954.

Zoosporangium flask-shaped or pyriform, embedded in the gelatinous sheath, zoospore cyst forming the apex of the somewhat elongated neck, 22–38 μ in total length by 8–16 μ in diameter, neck 4.8 μ in diameter, wall smooth, colorless, slightly thickened; rhizoids several, short, unbranched; zoospores globose or ovoid, 3.2–4.8 μ in diameter, with a posterior flagellum 8–16 μ long and an eccentric colorless refractive globule 1–1.6 μ in diameter, the first ones to emerge resting in a cluster at the orifice before assuming motility, the later ones emerging singly and swimming away at once, movement hopping; resting spore immersed in the gelatinous host sheath, globose, with a long, broad, persistent germ tube, terminated by the somewhat wider and thicker walled zoospore cyst, with a thick, smooth, hyaline, 2.5–3 μ wall, 13–21 μ in diameter, contents finely granular, 8–15 μ in diameter, with a large eccentric globule, apparently asexually formed, germination not observed.

Parasitic in ? *Gloeodinium sp.* (det. G. M. Smith), Smith's Bog, Cheboygan Co., Michigan, July 3, 1954.

A NEW SPECIES OF BLYTTIOMYCES

Blyttiomyces contains at present two species, *B. spinulosus* (Blytt) Bartsch and *B. laevis* Sparrow, the latter recently described from the Douglas Lake region (Sparrow, **1952**). We wish to record here the presence in this same region and in the Upper Peninsula of a third, strikingly distinct from other congeneric forms and indeed from all other members of the Phlyctidiaceae by the presence on the sporangial wall of a series of spiral thickenings. Unfortunately, the fungus occurred in exceedingly small numbers on pollen and could not be extensively multiplied. Hence, few developmental stages were found.

Mature sporangia were ovate to globose, with a rounded, refractive apiculus and one, occasionally two, basal papillae (FIG. 22). The endobiotic system consisted of an apophysis from which tapering branched rhizoids arose. The wall of the sporangium was hyaline in the immature stages, becoming light brown at maturity, and was ornamented with six to seven rows of helical bands which were occasionally once-branched in the lower portion of the sporangium. Sporangial discharge was observed in only a few instances. The globose zoospores, each with an eccentric refractive globule and posterior flagellum, initiated motility within the

sporangium, and upon deliquescence of the papilla emerged individually through the discharge pore and swam away immediately, with a rapid, darting movement (FIG. 23). Following zoospore discharge the sporangium remained erect and did not collapse. Endobiotic, smooth-walled resting spores were produced in much of the pollen used as bait. These were never seen to germinate, however, and hence the resting stage of the fungus cannot be described with certainty.

Blyttiomyces helicus sp. nov. FIGS. 22–23.

Zoosporangium late ovoideum vel globosum, 16–33.5 μ altum (apiculo crasso pallide brunneo, 2–2.5 μ alto, 4–6 μ lato, incluso), diametro 14–26.5 μ, muro pallide brunneo, incrassationes 6 vel 7 costiformes helicales 0.8–1 μ altas ex basi usque ad apiculum ferente. Pars endobiotica crassa ex apophyse 4–7 μ diam., axe rhizoidali diam. 2.1–3 μ, et ramis ultimis constans; zoosporis sphaericis 4–4.8 μ diam., globulo refractivo et flagello longo posteriori praeditis, in sporangio motilibus et ultime per porum solitarium (raro duos) basalem emergentibus, rapide saltuatim motitantibus. Sporae durantes non hucusque observatae.

Cultus in *Pinus* granulis pollinis, in *Spagno* et detrito, ad locum palustrem "Livingston Bog" dictum, Cheboygan Co., Mich., 8 Jul., 1954; in granulis *Pinus* effetis ad lacum "Cabin Lake," Chippewa Co., Mich., 23 Jul. 1954.

Zoosporangium broadly ovate to globose, 16–33.5 μ high, including the apiculus, by 14–26.5 μ in diameter, wall light brown and bearing six to seven narrow, 0.8–1 μ high, helical bands which extend from the base to the thick, light brown, 2–2.5 μ high by 4–6 μ in diameter, apiculus; endobiotic part coarse and extensive, consisting of a 4–7 μ in diameter apophysis from which emerges basally a rhizoidal axis 2.1–3 μ in diameter which branches distally; zoospores spherical, 4–4.8 μ in diameter, with an eccentric, colorless refractive globule 1.6–2 μ in diameter and a long posterior flagellum, moving in the sporangium before escaping through one, occasionally two, basal or subbasal discharge pores, movement a rapid darting; resting spores not observed.

On pine pollen bait, in *Sphagnum* and debris, Livingston Bog, Cheboygan Co., July 8, 1954; decaying pine pollen, Cabin Lake, Chippewa Co., Michigan, July 23, 1954.

A NEW SPECIES OF CHYTRIDIUM

A fourth new chytrid, belonging to *Chytridium*, was found on *Oedogonium sp.* from a boggy lake in Chippewa County, Upper Peninsula, growing associated with *Potamogeton confervoides* and *P. oakesianus*. Its most distinctive feature is the presence on the apical operculum of a prolonged solid mucro, resembling in this respect the diatom

parasite *C. perniciosum* Sparrow (**1933**). In contrast to the latter species, however, the new form has an endobiotic apophysis.

Observations on stages in the development of this chytrid indicate that the zoospore comes to rest and encysts on the host cell, puts forth a slender germ tube which penetrates the wall of the algal cell, and enlarges within it to form the more or less spherical apophysis (FIG. 24). The delicate, branched rhizoids subsequently arise from several points on the apophysis and ramify throughout the host cell. Thus, the apophysis is a primary structure and not laid down after the rudiments of the rhizoidal system. Prior to enlargement of the zoospore cyst the apophysis contains numerous large granules which in later stages of thallus development are carried into the sporangial rudiment. As the epibiotic body of the encysted zoospore expands to form the zoosporangium, its basal part becomes distinctly thick-walled in contrast to the more distal regions. Developing sporangia are at first broad pyriform to ovate in shape but soon develop the characteristic mucronate apiculus which in final stages of development becomes an operculum composed of solid wall material. The protoplasm of the immature sporangium remains finely granular for a considerable period during development. After all the contents of the endobiotic part have been transported to the rudiment of the sporangium and the latter has reached full size the characteristic refractive globules are organized in the contents. These, at first few in number, increase until about twenty are visible (FIG. 25). Spore cleavage then ensues. Finally the operculum dehisces, remaining attached to the sporangium at one side, and the zoospores emerge in a single amoeboid mass. They remain quiescent for a few minutes at the orifice of the sporangium before individuals attempt to extricate themselves from the group (FIG. 26). During this process, it is evident that the flagella of the spores are intricately entangled and that they extend back into the sporangium. Only after much tugging does a zoospore become free, after which it swims away with a darting movement. The empty sporangium does not collapse but remains intact with the operculum attached to one side and the basal, thicker walled part, clearly visible (FIG. 27).

Chytridium mucronatum sp. nov. FIGS. 24–27.

Zoosporangium ovoideum vel pyriforme, sessile vel brevissime stipitatum, 17.5–23.8 μ altum, 11.2–15.4 μ diametro, ad apicem operculo solido mucronato 5–6 μ alto, 5 μ diam. praeditum, membrana laevi hyalina, tenui sed prope basem et quoad partem zoosporae reliquam paulum incrassata. Pars endobiotica ex stipite in apophysin 5–9.8 μ diametientem globosum expanso et rhizoideis abundanter ex

apophyse per cellulam ramificantibus constans; zoosporis operculo dehiscente primum in massam amoeboidalem cohaerentibus quiescentibus, demum saltuatim et separatim motitantibus, globosis, 4.5–5.6 μ diam., globulum eccentricum 1–1.5 μ diam. et granula numerosa includentibus. Flagellum 24–28 μ longum. Sporae durantes haud observatae.

Parasiticum in *Oedogonii* speciebus in lacu dicto "Cabin Lake," Chippewa Co., in peninsula superiore Michiganensi, 23 Jul. 1954.

Zoosporangium ovate or pyriform, sessile or with a very short stalk, 17.5–23.8 μ high by 11.2–15.4 μ in diameter, the apex terminated by a mucronate, solid operculum, 5–6 μ high by 5 μ in diameter, wall smooth, colorless, thin except at the base where the remnants of the zoospore cyst persist; endobiotic system consisting of a short stalk which broadens into a globose apophysis 5–9.8 μ in diameter from which emerge numerous, delicate, branched rhizoids which ramify throughout the host cell; zoospores emerging in an amoeboid mass after dehiscence of the operculum and remaining quiescent for a few minutes, then swimming away with a hopping movement, globose, 4.5–5.6 μ in diameter, containing an eccentric, colorless, refractive globule 1–1.5 μ in diameter and numerous minute granules, flagellum 24–28 μ in length; resting spore not observed.

Parasitic on *Oedogonium sp.*, Cabin Lake, Chippewa Co., Upper Peninsula, Michigan, July 23, 1954.

NEWLY RECORDED SPECIES

OLPIDIUM ENDOGENUM (Braun) Schroeter

In *Pleurotaenium sp.* and *Cosmarium sp.*, small pond on road between Cross Village and Sturgeon Bay, Emmet Co., July 1, 1954 (coll. R. M. Johns).

Not previously reported in *Pleurotaenium* from the United States.

OLPIDIUM ENTOPHYTUM (Braun) Rabenhorst

In oospores of *Oedogonium sp.*, Nichol's Bog, *Bulbochaete sp.*, pond on road between Cross Village and Sturgeon Bay, July 1, 1954 (coll. and det. R. M. Johns).

Not previously reported in either host from the United States. As previously indicated (Sparrow, **1943**) this is a collective species.

OLPIDIUM HYALOTHECAE Scherffel

In *Desmidium schwartzii* (det. G. M. Smith), Smith's Bog, Nichol's Bog, July 3, 1954 (coll. and det. R. M. Johns).

This is the first record of the species in the United States.

OLPIDIUM SACCATUM Sorokin

In *Cosmarium sp.*, Smith's Bog, July 3, 1954 (coll. M. E. Barr, det. F. K. S.).

This species has not been previously reported from the United States.

OLPIDIUM GREGARIUM (Nowak.) Schroeter

In rotifer (?) eggs, Nichol's Bog, June 24, 1954 (coll. H. E. Clark, det. F. K. S.).

Reported twice before from the United States (Karling, **1941**, **1948**).

ROZELLA MONOBLEPHARIDIS-POLYMORPHAE Cornu

Parasitic on *Monoblepharis macrandra*, swamp, south end of Carp Lake, July 1, 1954 (coll. R. M. Johns, det. F. K. S.).

Our material differed from Cornu's in that only oogonia were parasitized, not the hyphae. Some hypertrophy of the host was noticeable. The zoospores were very minute, with several granules in the body and a single posterior flagellum. If the present form does relate to Cornu's species, and we think it does, this is the first report of zoospores and the first recorded occurrence in this country.

PHLYCTOCHYTRIUM AURELIAE Ajello

On purified shrimp skeleton bait, debris from under ice, Bryant's Bog, April 8, 1954 (coll. F. K. S., R. A. Paterson, R. M. Johns).

Not recorded since original description.

PODOCHYTRIUM CLAVATUM Pfitzer

On *Pinnularia sp.*, Livingston Bog, July 8, 1954 (coll. and det. M. E. Barr).

Not previously recorded on this host from the United States.

CHYTRIDIUM OLLA Braun

On oospores of *Oedogonium sp.*, Nichol's Bog, Cross Village pond, Emmet Co. (coll. and det. R. M. Johns), Smith's Bog, August 16, 1954 (coll. and det. M. E. Barr).

Not previously recorded from the United States.

ZYGORHIZIDIUM WILLEI Löwenthal

On filaments of *Zygnema sp.*, Nichol's Bog, June 24, 1954 (coll. M. E. Barr, det. F. K. S.).

Not previously reported from the United States.

NEPHROCHYTRIUM APPENDICULATUM Karling

On moribund cells of *Chara sp.*, Douglas Lake at depth of 4 m., June 25, 1954 (coll. R. A. Paterson, det. R. M. Johns).

NOWAKOWSKIELLA RAMOSA Butler

On nucules of moribund *Chara sp.*, Douglas Lake at depth of 4 m., June 25, 1954 (coll. R. A. Paterson, det. R. M. Johns).

BLASTOCLADIA PRINGSHEIMII Reinsch

On twigs, cedar thicket, south side of Carp Lake, July 1, 1954 (coll. R. M. Johns, det. F. K. S.).

GONAPODYA PROLIFERA (Cornu) Fischer

On twigs, cedar thicket, south shore of Carp Lake, July 1, 1954 (coll. and det. R. M. Johns).

Gametangial plants were found.

GONAPODYA POLYMORPHA Thaxter

On twigs, cedar thicket, south shore of Carp Lake, July 2, 1954 (coll. R. M. Johns, det. F. K. S.).

Gametangial plants were also found in this species. These are the first reports of gametangia since their discovery by Johns and Benjamin (**1954**).

MONOBLEPHARIS POLYMORPHA Cornu

On birch twigs, swamp, south side of Carp Lake, July 2, 1954 (coll. and det. F. K. S.).

APHANOMYCOPSIS BACILLARIACEARUM Scherffel

In a pennate diatom, Smith's Bog, July 3, 1954 (coll. M. E. Barr, det. F. K. S.).

RHIPIDIUM AMERICANUM Thaxter

On twigs, cedar thicket, south shore of Carp Lake, July 1, 1954 (coll. R. M. Johns, det. F. K. S.).

MINDENIELLA SPINOSPORA Kanouse

On *Amelanchier* fruits as bait in twig culture, Ocqueoc Lake, Presque Isle Co., July 26, 1954 (coll. and det. R. M. Johns).

MYZOCYTIUM PROLIFERUM Schenk

In *Closterium sp.*, planktonic in Devereaux Lake, July 28, 1954 (coll. and det. R. A. Paterson).

MYZOCYTIUM MEGASTOMUM de Wildeman

In *Closterium sp.*, occurring in the same cell as *M. proliferum*, planktonic in Devereaux Lake, July 28, 1954 (coll. and det. R. A. Paterson).

LAGENIDIUM CLOSTERII de Wildeman

In *Closterium sp.*, Nichol's Bog, August 12, 1954 (coll. and det. F. K. S.).

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NEW SPECIES OF GALERINA [1]

ALEXANDER H. SMITH AND ROLF SINGER [2]

This paper is a continuation of investigations on *Galerina*. Smith (**1953**) previously accounted for 28 species and discussed the monograph of the genus upon which we are engaged jointly. The present contribution contains 29 additional previously undescribed species.

During the season of 1953 we carried out a vigorous collecting program in northern Michigan with the University of Michigan Biological Station at Douglas Lake as headquarters. A good season was experienced and much information on *Galerina* obtained. Most important, however, we found that the important microscopic characters, in particular those relating to spore ornamentation, which we had emphasized as a result of our studies on dried material, were also readily demonstrable and constant on the fresh material. The results of that season's work have served to strengthen our species concepts as embodied in the previously undescribed taxa included here. Extensive field work in the western United States during the season of 1954 failed to turn up much in the way of galerinas that we had not had previously. However, the season, following a cold summer as it did, was not one of the best mushroom seasons for *Galerina*.

We wish, again, to express our appreciation to all who have aided our study, particularly to Prof. E. B. Mains, director of the University of Michigan Herbarium, for publication funds to defray the cost of publishing this paper out of turn in Mycologia. Financial assistance from the National Science Foundation, Washington, D. C., Mr. Wm. B. Gruber of Portland, Oregon, and the University Herbarium supported the extensive field program of 1954. The senior author wishes also to express his appreciation to Superintendent Preston P. Macy of Mt. Rainier National Park, for continued courtesies in connection with his work there. It is a pleasure for both of us to acknowledge the excellent cooperation of Dr. A. H. Stockard, director of the University of Michigan Biological Station, in making the facilities of the Station available to us

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[2] Studies from the University of Michigan Herbarium and Department of Botany, University of Michigan, Ann Arbor, Michigan and the Instituto Miguel Lillo, Tucumán, Argentina.

throughout the mushroom season. Many of the earlier collections cited in this paper were made on expeditions financed in a large measure by the Faculty Research Fund of the University of Michigan. Support from this fund was a major item in furthering the studies on *Galerina*.

1. **Galerina dimorphocystis** sp. nov.

Pileo 4–15 mm lato, ochraceo-brunneo, glabro; lamellis dilute ochraceo-brunneis; sporis levibus vel sublevibus, 7.5–10(–11) × (3.7–)4.5–6(–6.7) μ; pleurocystidiis nullis; stipite pallide brunneo vel dilute flavido vel pallidisimo; velo nullo; hyphis defibulatis. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *41401*.

Pileus 4–12(–15) mm broad, obtusely conic or campanulate with a straight margin, expanding to broadly conic or convex, often somewhat umbonate, surface moist and hygrophanous, when young often ochraceous tawny (*Yucatan*),[3] then pale ochraceous tawny, fading to pale pinkish buff, outer half translucent striate moist, densely pubescent when young, glabrescent; flesh very delicate, membranous, taste faintly raphanoid, odor similar or none; lamellae close to distant, broad (about 1.5 mm) to relatively narrow, ascending, pale delicate ochraceous tawny, adnate, edges even; stipe 15–30 mm long, 0.5–2 mm thick, hyaline to slightly dingy yellowish (*sombrero*), pruinose or appearing fibrillose below because of elongated caulocystidia (no veil present), midportion glabrescent, very delicate and brittle.

Spores 7.5–10(–11) × (3.7–)4.5–6(–6.7) μ, somewhat inequilateral in side view, more or less narrowly ovate in face view, minutely punctate to practically smooth, with normally thickened wall for the genus (not collapsing), plage area not differentiated; basidia 22.5–24 × 6–6.2 μ, 4-spored or rarely 2-spored; pleurocystidia none; cheilocystidia hyaline to yellowish, (22–)25–42 × 4.5–10.5 μ, rarely some 30–40 × 10–16 μ, capitate to subcapitate, the capitulum 3.7–6 μ, back of this the narrowest part of the neck 1.5–2.3(–3) μ thick, some of those on the part of the gill edge nearest the cap margin typically vesiculose and (7.7–)15–20 μ in diam. and some of these with an apical projection; hyphae of pileus trama (and hypodermium) often of short broad cells up to 38 μ wide, with incrusted pigment dissolving somewhat in KOH to color the mount yellow; pilocystidia in young caps numerous, capitate to subcapitate, up to 75 × 7.5 μ; clamp connections absent.

Habit, habitat and distribution. Gregarious on moss, especially over mossy logs in bogs or wet hillsides, early June to August. It was found abundantly in the vicinity of the lower falls of the Tahquamenon River in Tahquamenon Falls State Park, Mich., during 1953.

[3] Color names within quotation marks are taken from Ridgway (**1912**); those underlined are taken from Maerz and Paul (**1930**).

Material examined. Singer *N-117; N-266; N-267; N-532* (F); Sm-*41270; 41272; 41277; 41279; 41280; 41398; 41400; 41401*, TYPE; *41435; 41436; 41438; 41442; 41445; 41609; 41610; 41611; 41612; 41613; 41614; 41615; 41616; 41617; 41619; 41643.*

Observations. This is typically a small species likely to be mistaken for *G. hypnorum* in the field. The nearly hyaline stipe and lack of a veil distinguish it from *G. semilanceata* which, in addition, is typically more robust. The large vesiculose cheilocystidia are most numerous near the cap margin, but cannot always be found readily enough to make their presence a quick means of spot identification. However, they are significant in the delimitation of this species from *G. graminea*, which also has numerous pilocystidia. *G. dimorphycystis* differs from *G. brunneimarginata* in consistently smaller size, thicker-walled more conspicuously roughened spores, and different habitat. In some collections such as Sm-*41400* and Singer *N-266* there is a tendency toward the production of abnormally long, narrow (to $13 \times 4.5\,\mu$) spores which are drawn out to the apex, and which sometimes appear subangular in face view. These have been observed as both "diads" and "tetrads" in mounts—indicating that they may be produced on either 2- or 4-spored basidia. The ornamentation is always very fine, but varies from distinct to faint in a single print.

2. **Galerina subceracea** sp. nov.

Pileo 8–10 mm lato, cinnamomeo vel ochraceo-brunneo, canescente, opaco; lamellis pallidis dein brunnescentibus; sporis 8–10 × 5–5.5 μ, levibus; pleurocystidiis nullis; stipite carneo-alutaceo; velo subtiliter fibrilloso; hyphis defibulatis; ad muscos. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *43847*.

Pileus 8–10 mm broad, broadly conic with a bent-in margin, surface canescent but moist and hygrophanous beneath the canescence, pale "cinnamon" to nearly "ochraceous tawny," fading evenly to "pinkish buff," opaque when moist, glistening when faded; flesh thin but firm and with a waxy feel, pale yellow, odor and taste none; lamellae distant, thick, broad, adnate to adnexed, *pallid* becoming pale ochraceous-cinnamon, edges uneven; stipe 20–40 mm long, 1.5–2 mm thick, equal, hollow, fragile, near pinkish buff over all or tinged cinnamon above, thinly coated with white fibrils from a slight veil which scarcely leaves a zone.

Spores 8–10 × 5–5.5 μ, obscurely inequilateral in side view, ovate in face view, smooth or very faintly rough, very pale in Melzer's reagent, pale yellow in water when fresh, ochraceous in KOH; basidia 4-spored; pleorocystidia none but some basidioles with ochraceous content in KOH; cheilocystidia 26–34 × 6–10 μ, ventricose with a narrow neck and sub-

capitate apex, neck about 3 μ in diam. below the capitulum, a few merely with subacute apices; gill trama fulvous in KOH; pileus trama homogeneous, rusty fulvous in KOH, cuticle only slightly differentiated; clamp connections absent.

Habit, habitat and distribution. Scattered on moss in a swampy area, Wilderness Park, Mich., Oct. 7, 1953. Known only from the type locality.

Material examined. Sm-*43847*, TYPE.

Observations. The canescent pileus, wider spores, thick, waxy gills, and cinnamon color, along with the presence of a veil, appear to distinguish this from *G. brunneimarginata*. Observations on the spores must be made with exceptional care. The granular content becomes aggregated against the wall, producing the illusion of roughness, and occasionally these contents become separated by a hyaline homogeneous area (a diffuse layer of some liquid?) which, if the area coincides with the plage area in the view you happen to get, will create the illusion that a smooth plage is present bounded by minute granules of roughness. However, by careful focusing on material mounted in Melzer's reagent this artifact need cause no confusion.

3. **Galerina turfosa** sp. nov.

Pileo 8–15 mm lato, convexo, ferrugineo-castaneo ochraceo-brunneo, margine primum stricto; lamellis adnatis vel subdecurrentibus, latis; sporis 10–12(–13) × 6.5–7.5(–8) μ, irregulariter rugulosis; pleurocystidiis nullis; stipite 20–60 mm longo, 2–3 mm crasso, primum veli relictis obsito, glabrescente; odore nullo vel subtili; sapore subfarinoso; hyphis fibulatis; inter *Sphagna* atque alios muscos. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *33-1101*.

Pileus 8–15 mm broad, convex with a straight margin when young, broadly convex in age, margin at first fringed with scattered fibrils from the rudimentary veil, otherwise glabrous, when fresh lubricous and "Sanford's brown" to "chestnut" (ferruginous to bay), slowly changing to "ochraceous tawny" in age before fading, fading to ochraceous buff; flesh thin, fragile and watery, odor none or slight, taste slightly farinaceous; lamellae broad, adnate to subdecurrent, broadest at stipe, (3–4 mm), tapering to edge of cap, subdistant, dull brown young, cinnamon-brown in age, margin white floccose. Stipe 20–60 mm long, 2–3 mm thick at apex, equal, fragile, hollow, base slightly bulbous, pale ochraceous, at first covered over lower half by scattered fibrils representing remnants of the veil, glabrescent.

Spores 10–12(–13) × 6.5–7.5(–8) μ, inequilateral in side view, broadly ovate in face view (many almost angular-ovate), outer wall sep-

arating around suprahilar depression to produce loosened wrinkled blisters, apical callus present; basidia 4-spored, 28–34 × 8–9(–10) μ, hyaline in KOH; pleurocystidia none or rare and then near the gill edge; cheilocystidia (36–)40–50 × 9–12 μ, fusoid-ventricose to subcylindric, apices obtuse, walls thin and hyaline in KOH; pileus trama homogeneous beneath a poorly formed cuticle of subgelatinous narrow hyphae (no well differentiated pellicle); clamp connections present.

Habit, habitat and distribution. Gregarious among *Sphagnum* and other mosses, Whitmore Lake, Mich., September, 1933; known only from the type locality.

Material examined. Sm-*33-1101*, TYPE; Sm-*33-1022*.

Observations. The ferruginous color of the pileus in young specimens and the cinnamon-brown gills at maturity distinguish this species in the field from others of the *G. cerina* complex. Under the microscope the large almost angular-ovate spores are somewhat distinctive. The habitat was a burn which at its edges had experienced quite an overgrowth of mosses, including *Sphagnum*, and it was in this zone that both collections were made.

4. **Galerina fallax** sp. nov.

Pileo 5–10(–15) mm lato, ochraceo-brunneo, glabro nudoque; lamellis ascendentibus, subliberis, latis; stipite gi[illegible]allide ochraceo ad apicem, ochraceo-brunneo ad basin; sporis 7–9 × 5–6 μ, calyptratis; cheilocystidiis brevibus angustisque; hyphis fibulatis; ad truncos muscosos coniferarum. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *16239*.

Pileus 5–10(–15) mm broad, obtusely conic becoming broadly conic to nearly convex, or remaining conic with a flaring margin, surface glabrous, moist and hygrophanous, "tawny" to "ochraceous tawny" and conspicuously translucent striate moist, fading to near cinnamon buff but darkening in drying, at first the disc with a pale watery circumscribing line; lamellae ascending, nearly free, close to nearly subdistant, broad, edges even; stipe 10–30 mm long, filiform or nearly so, equal, tubular, fragile, glabrous, pale ochraceous above, ochraceous tawny or darker below.

Spores 7–9 × 5–6 μ, inequilateral in side view, ovate in face view, tawny or darker in KOH, exosporium loosening and separable from episporium at least over basal portion, apical callus present; basidia 4-spored, 17–20 × 6–7 μ, hyaline in KOH; pleurocystidia none; cheilocystidia subventricose with obtuse apices or subcylindric, 24–35 × 6–9 μ, hyaline, thin-walled, smooth, gill trama interwoven to subparallel, yellow

from incrusting pigment; pileus trama homogeneous, ochraceous tawny or darker from incrusting pigment; clamp connections present.

Habit, habitat and distribution. Gregarious on mossy conifer logs, Shuksan Inn, Wash. Also known from Michigan and New York. It fruits during the late summer and fall.

Material examined. Sm-*16239*, TYPE; Sm-*33-207; 33-626; 33-952; 25513; 29193; 29251; 36331; 36380; 36400; 36402; 36545; 36742; 36785; 36879; 36887; 36900; 37039; 39042; 39266; 40998; 41138; 41090; 41157; 41253; 41266; 41393; 41434; 41896; 42588; 42904.* Kauffman & Smith, 9–22–20, Rock River, Mich. (as *G. hypnorum*).

Observations. This is a common fungus on mossy logs and is one of the segregates of the old "*Galera hypnorum*." *Galerina hypnorum* in this work is characterized by consistently larger, non-calyptrate spores. The separation of the exosporium to form blisters or loose areas can be demonstrated in both fresh and dried material. For all practical purposes there is no veil.

5. **Galerina acicola** sp. nov.

Pileo 10–20 mm lato, obtuse conico, cinnamoneo-brunneo; lamellis ochraceo-brunneolis; stipite 40–50 mm longo, 2–2.5 mm crasso, pallide brunneo ad apicem et aquose cinnamomeo-brunneo ad basin, veli vestigiis obtecto; sporis 11–13 × 6–7 μ; cheilocystidiis 46–60 × 7–12 μ, ampullaceis et interdum subcapitatis; pleurocystidiis nullis; hyphis fibulatis; ad acus coniferarum, Oregon. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *24101*.

Pileus 10–20 mm broad, obtusely conic, expanding to conic-campanulate, surface glabrous except for a few evanescent fibrils along the margin from the thin partial veil, moist and hygrophanous, "cinnamon-brown" on disc, dull "ochraceous tawny" over striate marginal area, fading to pale tan or darker; flesh concolorous with the pileus surface, thin, fragile, odor and taste not distinctive; lamellae bluntly adnate to slightly adnexed, broad, close to subdistant, dull ochraceous tawny (concolorous with cap margin), edges even to minutely crenulate; stipe 40–50 mm long, 2–2.5 mm thick, equal, sordid watery cinnamon-brown below, pallid brownish above, not darkening much more in aging, lower portion at first with scattered white fibrillose patches representing veil remnants, apex slightly pruinose.

Spores 11–13 × 6–7 μ, slightly inequilateral in side view, in face view narrowly ovate, surface appearing smooth in many but in others the exosporium separating from episporium forming distinct blisters or separating in small sheets, suprahilar depression smooth, apical callus present; basidia 4-spored, 26–30 × 9–10 μ, hyaline in KOH; pleuro-

cystidia none seen; cheilocystidia abundant, 46–60 × 7–10(–12) μ, narrowly ventricose with greatly elongated necks and obtuse apices or the latter almost subcapitate, hyaline and thin-walled in KOH; gill trama somewhat interwoven, the walls yellow to tawny with incrusting pigment; pileus trama homogeneous tawny revived in KOH because of incrusting pigment; clamp connections present.

Habit, habitat and distribution. Gregarious on conifer needles, Little Crater Lake, Mt. Wood Nat. Forest, Ore., Oct. 5, 1946. Known only from the type locality.

Material examined. Sm-*24101*, type.

Observations. This species grows on the naked needle beds in the manner of *Mycena psammicola*, i.e. scattered in abundance over a considerable area. In addition to this distinctly different habit and habitat, it differs from *G. cerina* in having larger spores on 4-spored basidia, and the stipe is consistently darker at the base than at the apex though in age it does not become bister. In most of the variants of *G. cerina* this character is not so well developed and does not show on young or freshly matured carpophores.

6. **Galerina cerina** sp. nov.

Pileo 5–15 mm lato, conexo vel subconico, glabro, lubrico, intense ochraceo-brunneo vel ad marginem, subpallidiore hydrophano, in statu sicco alutaceo-coriicolori; odore saporeque haud notabilibus; lamellis latis, adnatis, subdistantibus, pallide ochraceo-alutaceis, dein ochraceo-brunneis; stipite 20–30(–50) mm longo, 2–3 mm lato, ochraceo-brunneo, ad apicem lamellis concolori, subtiliter velato; sporis 8.7–12 × 5.5–7 μ, calyptratis; cheilocystidiis 30–40(–50) × 7–12 μ, fusoideo-ventricosis vel ventricoso-subcapitatis; hyphis fibuligeris. Inter Polytricha, rarius inter Sphagna, Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *1188*.

KEY TO VARIETIES AND FORMS

1. Veil pale yellow ..... var. *luteovelata*
1. Veil pallid to pure white 2
   2. Cheilocystidia 33–34 × 10–15 μ; spores 8–10 × 5–6.5 μ ..... var. *brachycystis*
   2. Cheilocystidia either longer or narrower 3
3. Cheilocystidia most ventricose-subcapitate, 30–40(–50) × 7–12 μ; spores 8.7–12 × 5.5–7 μ; mostly on *Polytrichum*, rarely on *Sphagnum* ..... var. *cerina*
3. Not with all the above characters; not on *Polytrichum* 4
   4. Spores relatively small, 8.3–10.3 μ long; cheilocystidia 31.5–58 × 5.3–10 μ, with elongated neck, subcapitate and then thinnest portion of neck 2.2–3.8 μ thick, more often non-capitate ..... var. *ampullicystis*
   4. Spores relatively larger, 9–15 μ long; cheilocystidia mostly not as above .. 5
5. Cheilocystidia irregular in shape, often with outgrowth from capitulum, or neck with irregular swellings; pileus obtuse ..... var. *contorticystis*

5. Cheilocystidia not so; pileus often acute.....6
6. Cheilocystidia 24–36(–39) × 6.5–9.7 μ.....var. *bresadolae*
6. Cheilocystidia 26–45(–80) × 5.2–12(–15) μ.....var. *longicystis*

GALERINA CERINA var. **cerina**

Pileus 5–15 mm broad, convex to subconic, glabrous, lubricous, margin striatulate, color when moist evenly rich "tawny" or the margin slightly paler, fading to buffy tan, margin at very first with a slight fringe of fibrils; fresh relatively thick and watery for such a small carpophore, concolorous with surface, odor and taste not distinctive; lamellae broad, adnate, subdistant, pale ochraceous buff becoming ochraceous tawny, edges even; stipe 20–30(–50) mm long, 2–3 mm thick, equal, fragile, undulating, tubular, apex concolorus with young gills, usually "tawny" over lower portion or becoming so and there at first covered by the remains of the slight pallid veil, naked below in age, apex pruinose.

Spores 8.7–12 × 5.5–7 μ, inequilateral in side view (5.5–6 μ broad), in face view ovate and very slightly broader (slightly lentiform), "tawny" in KOH under the microscope, smooth but exosporium separating from around the plage area to give it a ragged boundary and in a fair percentage in each deposit showing further separation in the form of blisters or separating completely around the apiculus to give a sack-like effect; basidia 27–3.5 × 8–10 μ, 4-spored; pleurocystidia none; cheilocystidia 30–40(–50) × 7–12 μ, some fusoid-ventricose and many ventricose-subcapitate; gill trama regular, tawny from incrusting pigment; pileus trama homogeneous, tawny in KOH; clamp connections present.

Habit, habitat and distribution. Gregarious on *Polytrichum* or more rarely on *Sphagnum*, sometimes on humus in *Sphagnum* bogs or in burned areas from Eastern North America to the Pacific Coast. More common in the spring and early summer than in the fall.

Material examined. Singer *N-1325* (CF); *N-1249* (CF); Cornell U. Herb. no. *25019*-TYPE *Galerula cerina;* Sm-*33-606; 1185* (with elongated cheilocystidia); *1188*-TYPE; *1198; 1199; 28745; 33989; 36743; 36744* (spores narrow); *36747; 36776; 36784; 36800; 36967; 37050; 37332; 37756; 41246* (with some abnormal spores); *41247; 41248; 41249; 41250; 41251; 41252; 41263; 41271; 41275; 41282; 41305* (2 types of spores); *41306; 41307; 41309* (abnormal spores); *41338; 41391; 41394; 41395; 41433; 41443; 14521; 41618; 41621; 41664* (no veil); *41674; 41692; 42001; 42008; 42010; 42011; 42012; 42019; 42020; 42041; 42154; 42159; 42160; 42162; 42164; 42183* (near var. *brachycystis*); *42201; 42202; 42204; 42205; 42269; 42909; 42920; 43010; 43026; 43916; 43920; 44036.*

Observations. There is a strong tendency for pleurocystidia to be produced wherever the hymenium has been injured.

GALERINA CERINA var. CERINA f. **bispora**

A varietate *cerina* f. *cerina* basidiis bisporis, sporis 11–14 × 6.5–8 μ, et pileo acuto differt. Specimen typicum in Herb. Univ. Mich. conservatum: Smith n. *44136*.

Habit, habitat and distribution. On *Polytrichum*, near Whitemore Lake, Mich., June 1951, Smith n. *44136*.

Observations. In the dried condition, the pilei do not appear to be much different from those of the 4-spored form, but there did seem to be fewer capitate cheilocystidia. Further observations will show whether this, as we now assume, is merely a bisporous form of the type variety, or a variety in its own right.

GALERINA CERINA var. **ampullicystis** var. nov.

A varietate *cerina* cheilocystidiis longioribus apice tenioribus vel magis capitatis differt; ad *Sphagnum* et muscos alios sed non *Polytrichum* ad ligna putrida muscosa. Specimen typicum in Herb. Chicago Nat. Hist. Mus. conservatum; Singer n. *142*.

Pileus 2–6 mm broad, up to 5 mm high, obtusely conic to campanulate-umbonate, not acute, at times becoming convex, glabrous, moist, hygrophanous, translucent-striate and tawny when moist, paler between the striae and sometimes also on the umbo, fading from umbo outward, buff when faded, margin straight at first; flesh very thin, fragile, inodorous; lamellae moderately broad, flat or almost ventricose, ascending, finally nearly horizontal, distant to subdistant, adnate, light ochraceous brown; stipe 10–30 mm long, 0.3–1 mm thick, base up to 1.5 mm in diam., equal or with a slight bulb, pruinose at the apex, otherwise with some appressed silky fibrils from the veil, sordid stramineous, often whitish at base, finally the base watery cinnamon brown; veil pallid to white.

Spores 8.3–10.3 × 5.3–6 μ (11–13 × 6–7 μ), calyptrate, somewhat inequilateral in side view, ellipsoid-subamygdaliform in face view, rarely a few with dorsal spur; basidia 20–27.5(–34.3) × 6–8(–10) μ, 4-spored, with very few to many 2-spored intermixed; pleurocystidia none; cheilocystidia ventricose, fusoid below, ampullaceous with a thin elongated neck, more rarely subcapitate and then thin portion beneath capitulum 2.2–3.8 μ in diam., 31–58 × 5.3–10 μ (if not capitate neck sometimes up to 5 μ broad), numerous vesiculose bodies 27 × 9 μ also present toward the pileus margin; pileus trama and epicutis not pigmented, not gelatinized, without pilocystidia; clamp connections present.

Habit, habitat and distribution. Among mosses (not *Polytrichum*) including *Sphagnum*, very frequently on mossy coniferous logs, in small groups or solitary, Michigan (Cheboygan and Luce counties).

Material examined. Singer *N-142*, TYPE, *N-530*, *N-1166*, *N-1322*, *N-1233b*; *N-1228*, (CF); Sm-*42176*.

Observations. This variety seems to approach *G. fallax* in its macroscopic characters but has a more pronounced veil and slightly larger spores, both of which are characters of *G. cerina*, to which we attach it as a variety. The tendency of the neck of the cheilocystidium to be very thin is seen here to be an inconstant character but the average cheilocystidium has a thinner neck than in var. *cerina*.

GALERINA CERINA var. **luteovelata** var. nov.

A varietate *cerina* velo flavo differt. Ad truncum Piceinum muscosum. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *13191*.

Pileus 8–10(–15) mm broad, obtusely conic, remaining unexpanded, striate to disc when moist, hygrophanous, "ochraceous tawny" with "tawny" striations and disc when moist, "cinnamon buff" faded, glabrous; flesh concolorous with surface, thin and fragile, odor and taste not distinctive; lamellae subdistant (9–11 reach the stipe), broad (2 mm), 1 row of lamellulae, adnate but soon seceding, near "clay color" young, soon "ochraceaous tawny," edges even; stipe 10–20 mm long, 1 mm thick, equal or base flaring a little, very fragile, "ochraceous buff" and fibrillose with yellow fibrils toward the base, paler and pruinose above, glabrescent in age.

Spores 10–12.5 × 6.5–8 μ, somewhat inequilateral in side view, ovate in face view, tawny or darker in KOH, smooth or exospore slightly rugulose and around the apiculus separating from epispore in blisters and thin sheets, plage smooth and with a ragged boundary; basidia 4-spored, 26–30 × 9–10 μ, hyaline in KOH; pleurocystidia none; cheilocystidia abundant, 30–42 × 7–12 μ, apices up to 9–12 μ broad in some and obtuse to broadly rounded, bases ventricose, hyaline, thin-walled, in many the broadly rounded apex wider than the basal ventricose portion; gill trama interwoven, pale rusty brown in KOH from incrusting pigment; pileus trama homogeneous, hyphae dark rusty brown from heavily incrusting pigment as revived in KOH; clamp connections present.

Habit, habitat and distribution. Scattered on mossy side of a dead spruce stub, Hoh River, Olympic National Forest, Wash., May 7, 1939.

Material cited. Sm-*13191*, TYPE; Sm-*42154*(?).

Observations. Collection Sm-*42154* has the large spores of var.

luteovelata and also a mixture of cheilocystidia with the very broad apices, but the veil was white. It was growing in a turf of *Dicranum* near the Lower Falls, Tahquamenon Falls State Park, Michigan. This form needs further study. The most interesting character involved here is the production of broadly rounded cheilocystidia but the character is not fixed to the extent that other types do not occur.

GALERINA CERINA var. **longicystis** var. nov.

A varietate *cerina* cheilocystidiis longioribus differt. Ad lignum putridum muscosum. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *40453*.

Pileus 4–8 mm broad and high, sharply conic or conic-campanulate and subpapillate, not expanding, glabrous, long-striate with translucent striations, hygrophanous, pale fulvous to fulvous but never as bright as "tawny" (between *raw sienna* and *Peruvian brown*) moist, paler between the striae, when faded pinkish buff to pale ochraceous; odor none; taste not distinctive; lamellae distant, broad, ventricose, ochraceous tawny, ascending adnate, edges even; stipe 20–30 mm long, 0.6–1 mm thick, equal, dull honey-yellow above and over all at first, becoming pale ochraceous tawny but not darkening appreciably from the base upward, with a thin coating of pallid velar fibrils over lower portion.

Spores 9–12.5 × 5.5–7.2 μ, ovate in face view, obscurely inequilateral in side view, rich tawny in KOH, wall appearing smooth but under oil slightly calyptrate and minutely areolately marked and with a ragged line delimiting the plage; basidia 4-spored; pleurocystidia none; cheilocystidia fusoid-ventricose with a ventricose lower portion and apices varying from subacute to oval-enlarged, 26–45(–80) × 5.2–12(–15) μ, neck at times 2.3–4.5 μ broad and short, capitulum up to 9 μ broad; gill trama somewhat interwoven; pileus trama homogeneous, dingy ochraceous in KOH; caulocystidia ventricose with gradually attenuate non-capitate apices, about 31–45 μ long; cuticle of pileus of narrow appressed non-gelatinous incrusted hyphae; clamp connections present.

Habit, habitat and distribution. Scattered on mossy logs, Carbon River, Mt. Rainier National Park, Washington, also in Michigan.

Material examined. Singer N-*1279*; Sm-*40453*, TYPE; Sm-*41660*; one unnumbered collection (Tahquamenon Falls State Park).

Observations. The spores from 4-spored basidia are slightly larger than in var. *ampullicystis*, but the cheilocystidia are not too dissimilar. However, the pileus of var. *longicystis* is sharply conic. One of the important characters, here, however, is that the spores are less calyptrate than in most other variants of *G. cerina*. This has influenced us to propose it as a variety.

GALERINA CERINA var. **bresadolae** var. nov.

A var. brachycysti sporis majoribus nec non cheilocystidiis minus crassis differt. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *40994*.

Pileus 6–12 mm broad at base, sharply conic to cuspidate and remaining unexpanded, surface glabrous, moist, hygrophanous, bright ochraceous tawny fresh, fading to yellowish (near warm buff); lamellae distant, ascending-adnate, narrow to moderately broad, ochraceous tawny or nearly so, edges even; stipe 25–40 mm long, 1 mm thick, equal, about pale honey color and most appreciably darker at the base, slightly fibrillose over lower half from remains of rudimentary veil.

Spores 9–12 × 6.5–5.8 μ, inequilateral in side view, in face view broadly ovate, tawny in KOH, smooth except for blisters or a loose sheath of outer layer bordering the suprahilar depression; basidia 4-spored; pleurocystidia none seen; cheilocystidia abundant, 24–36 × 6–10 μ, fusoid-ventricose with short necks and obtuse to subcapitate apices; gill trama somewhat interwoven; pileus trama homogeneous; clamp connections present.

Habit, habitat and distribution. Scattered on mossy logs, Green Lake, Mt. Rainier National Park, Wash., October.

Material examined. Sm-*40994*, TYPE.

GALERINA CERINA var. BRESADOLAE. Eastern variant

Pileus 2.5–8 mm broad, conic-cuspidate to campanulate, often but not consistently acute, glabrous and smooth, ochraceous brown to tawny (*raw sienna* to *gold pheasant*), but paler between the striae and sometimes also paler on umbo, hygrophanous, fading to ochraceous pallid, flesh inodorous; lamellae distant, ascending, ventricose, ochraceous brown; stipe 10–27 × 0.7–1.3 mm, equal or slightly tapering upwards and then reaching 1.8 mm at base in diameter, pruinate at apex, pale melleous to ochraceous pallid, unicolorous, with extremely fugaceous silky whitish veil in very young specimens.

Spores 9.8–12 × 5.3–7.5 μ, only slightly inequilateral, all or at least very many distinctly calyptrate; basidia (18–)25–33.5 × 7.5–9.7 μ, 4-spored (or intermixed with 2-spored ones); cheilocystidia 24–36(–39) × 4.5–12 μ, ventricose below, with constriction (3–4.5 μ diameter) underneath a subcapitate apex (3.5–7.5 μ), much more rarely non-capitate, hyaline; pleurocystidia none; pilocystidia none; caulocystidia present at apex of stipe; all hyphae with clamp connections.

Habit, habitat and distribution. On moss and among thin moss covers over very rotten wood, never on *Polytrichum* or *Sphagnum*, fruiting from June until August, Luce and Cheboygan Counties, Mich. (see also Observations).

Material examined. Singer *N-143, N-1174a, N-1233a, N-1323.*

Observations. The eastern variant differs very slightly from the western material in the more inequilateral shape of the spores and side view, a generally more obtuse pileus, and more ventricose lamellae.

What is probably the same variant was determined as *Galera cerina* by Bresadola (Atkinson Herb. Cornell n. *3956*). It was collected by Atkinson between 4000 and 5000 ft. in the Blue Ridge Mts., N. C., and has spores exactly as described above.

GALERINA CERINA var. **brachycystis** var. nov.

A var. *cerina* differt pileo conico, cinnamomeo-brunneo; cheilocystidiis (32–)34–40(–44) × 10–15 μ, obtusis. Specimen typicum in Herb. Univ. Mich conservatum; Smith n. *33-606.*

Pileus 3–5 mm broad, conic, remaining unexpanded, margin straight, glabrous, hygrophanous, cinnamon brown moist, becoming pale ochraceous tawny before fading, ochraceous to "ochraceous buff" faded, translucent striate to apex when moist; flesh very thin and delicate, odor and taste not distinctive; lamellae adnate, distant, moderately broad, pale ochraceous like the faded pileus, edges even; stipe 25–30 mm long, filiform, equal, with a small bulb at base, lower part with scattered appressed fibrils from the thin veil, upper part faintly pruinose, glabrous in age, pale watery brown over all.

Spores 8–10(–11) × 5–6.5 μ, inequilateral in side view, ovate to obscurely angular-ovate in face view, dull tawny as revived in KOH, the exospore loosening around the hilar region and partially separating from the epispore, with an indistinct apical callus; basidia 24–26 × 9–10 μ, 1-, 2-, 3-, 4-spored, spore size taken from a 2–4-spored cap, hyaline in KOH; pleurocystidia none; cheilocystidia abundant (32–)34–40(–44) × 10–15 μ, broadly fusoid-ventricose with short necks and obtuse apices, thin-walled, hyaline in KOH; gill trama interwoven, hyaline or nearly so in KOH; pileus trama homogeneous, pale yellow from incrusting pigment; clamp connections present.

Habit, habitat and distribution. Gregarious on mosses in moist shady places on sandy soil (often on *Polytrichum piliferum*), George Reserve, Pinckney, Michigan, July 21, 1933. Known only from the type locality.

Material examined. Sm-*33-606*, TYPE; Sm-*33-610.*

Observations. The short fat cheilocystidia and relatively small spores led us at first to regard this as a distinct species but in view of the variability of the cheilocystidia in *G. cerina* and the variable number of spores born on a basidium in the material cited above, it is very unlikely that in the material available we have a true picture of this variant and

GALERINA CERINA var. **bresadolae** var. nov.

A var. *brachycysti* sporis majoribus nec non cheilocystidiis minus crassis differt. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *40994*.

Pileus 6–12 mm broad at base, sharply conic to cuspidate and remaining unexpanded, surface glabrous, moist, hygrophanous, bright ochraceous tawny fresh, fading to yellowish (near warm buff); lamellae distant, ascending-adnate, narrow to moderately broad, ochraceous tawny or nearly so, edges even; stipe 25–40 mm long, 1 mm thick, equal, about pale honey color and most appreciably darker at the base, slightly fibrillose over lower half from remains of rudimentary veil.

Spores 9–12 × 6.5–5.8 μ, inequilateral in side view, in face view broadly ovate, tawny in KOH, smooth except for blisters or a loose sheath of outer layer bordering the suprahilar depression; basidia 4-spored; pleurocystidia none seen; cheilocystidia abundant, 24–36 × 6–10 μ, fusoid-ventricose with short necks and obtuse to subcapitate apices; gill trama somewhat interwoven; pileus trama homogeneous; clamp connections present.

Habit, habitat and distribution. Scattered on mossy logs, Green Lake, Mt. Rainier National Park, Wash., October.

Material examined. Sm-*40994*, TYPE.

GALERINA CERINA var. BRESADOLAE. Eastern variant

Pileus 2.5–8 mm broad, conic-cuspidate to campanulate, often but not consistently acute, glabrous and smooth, ochraceous brown to tawny (*raw sienna* to *gold pheasant*), but paler between the striae and sometimes also paler on umbo, hygrophanous, fading to ochraceous pallid, flesh inodorous; lamellae distant, ascending, ventricose, ochraceous brown; stipe 10–27 × 0.7–1.3 mm, equal or slightly tapering upwards and then reaching 1.8 mm at base in diameter, pruinate at apex, pale melleous to ochraceous pallid, unicolorous, with extremely fugaceous silky whitish veil in very young specimens.

Spores 9.8–12 × 5.3–7.5 μ, only slightly inequilateral, all or at least very many distinctly calyptrate; basidia (18–)25–33.5 × 7.5–9.7 μ, 4-spored (or intermixed with 2-spored ones); cheilocystidia 24–36(–39) × 4.5–12 μ, ventricose below, with constriction (3–4.5 μ diameter) underneath a subcapitate apex (3.5–7.5 μ), much more rarely non-capitate, hyaline; pleurocystidia none; pilocystidia none; caulocystidia present at apex of stipe; all hyphae with clamp connections.

Habit, habitat and distribution. On moss and among thin moss covers over very rotten wood, never on *Polytrichum* or *Sphagnum*, fruiting from June until August, Luce and Cheboygan Counties, Mich. (see also Observations).

Material examined. Singer *N-143, N-1174a, N-1233a, N-1323.*

Observations. The eastern variant differs very slightly from the western material in the more inequilateral shape of the spores and side view, a generally more obtuse pileus, and more ventricose lamellae.

What is probably the same variant was determined as *Galera cerina* by Bresadola (Atkinson Herb. Cornell n. *3956*). It was collected by Atkinson between 4000 and 5000 ft. in the Blue Ridge Mts., N. C., and has spores exactly as described above.

GALERINA CERINA var. **brachycystis** var. nov.

A var. *cerina* differt pileo conico, cinnamomeo-brunneo; cheilocystidiis (32–)34–40(–44) × 10–15 μ, obtusis. Specimen typicum in Herb. Univ. Mich conservatum; Smith n. *33-606.*

Pileus 3–5 mm broad, conic, remaining unexpanded, margin straight, glabrous, hygrophanous, cinnamon brown moist, becoming pale ochraceous tawny before fading, ochraceous to "ochraceous buff" faded, translucent striate to apex when moist; flesh very thin and delicate, odor and taste not distinctive; lamellae adnate, distant, moderately broad, pale ochraceous like the faded pileus, edges even; stipe 25–30 mm long, filiform, equal, with a small bulb at base, lower part with scattered appressed fibrils from the thin veil, upper part faintly pruinose, glabrous in age, pale watery brown over all.

Spores 8–10(–11) × 5–6.5 μ, inequilateral in side view, ovate to obscurely angular-ovate in face view, dull tawny as revived in KOH, the exospore loosening around the hilar region and partially separating from the epispore, with an indistinct apical callus; basidia 24–26 × 9–10 μ, 1-, 2-, 3-, 4-spored, spore size taken from a 2–4-spored cap, hyaline in KOH; pleurocystidia none; cheilocystidia abundant (32–)34–40(–44) × 10–15 μ, broadly fusoid-ventricose with short necks and obtuse apices, thin-walled, hyaline in KOH; gill trama interwoven, hyaline or nearly so in KOH; pileus trama homogeneous, pale yellow from incrusting pigment; clamp connections present.

Habit, habitat and distribution. Gregarious on mosses in moist shady places on sandy soil (often on *Polytrichum piliferum*), George Reserve, Pinckney, Michigan, July 21, 1933. Known only from the type locality.

Material examined. Sm-*33-606,* TYPE; Sm-*33-610.*

Observations. The short fat cheilocystidia and relatively small spores led us at first to regard this as a distinct species but in view of the variability of the cheilocystidia in *G. cerina* and the variable number of spores born on a basidium in the material cited above, it is very unlikely that in the material available we have a true picture of this variant and

hence, while it is important to put it on record in accordance with the principles we are following, we hesitate to dignify it with the rank of a species. We hesitate to refer it to *G. fallax* as a variety because the habitat is so typical of *G. cerina*.

GALERINA CERINA var. **contorticystis** var. nov.

Var. *cerinae* similis et pileus laete fulvus; sporae 10–12 × 6.8–7.5 μ; cheilocystidia multiformia. Specimen typicum in Herb. Chic. Nat. Hist. Mus. conservatum; Singer n. *1326*.

Pileus 5–8 mm broad, obtusely conic then campanulate and rounded on apex, rather intensely colored (*gypsy* to *copper*), in age more ochraceous tawny, young cap *persimmon*, not viscid, glabrous, with straight margin, transparently striate and also sulcate when mature, flesh inodorous; lamellae ochraceous brown, subdistant, very broad, somewhat ascending even when mature; stipe about 30 mm long, 1–1.7 mm thick at apex, evently *topaz*, eventually yellow (*golden corn*), with strong fibrils from the hyaline veil which still shows in mature specimens but has no tendency to become yellow as the stipe does.

Spores 9.8–12 × 6.8–7.5 μ, calyptrate; basidia 4-spored; cheilocystidia 27–44(–51) × 7–8 μ, extremely irregular and varying from subulate to ventricose below and subcapitate above, the capitulum often mucronate, often entirely flexuous with repeated constrictions, the subcapitate ones predominant, often budding, thinnest part of neck 2–4.5 μ, pleurocystidia none; clamp connections present.

Habit, habitat and distribution. Gregarious to scattered on moss over rotten conifer wood, Aug. 17, 1953, Tahquamenon Falls State Park, Luce Co., Mich.

Material examined. Singer, N-*1326*, TYPE.

Observations. The intensive color of the pileus and its sulcate striations at maturity, the stipe with its tendency to become yellow, and the curiously shaped cheilocystidia are distinctive. In quite a few of the collections of *G. cerina* we have noticed varying numbers of this curious type of cheilocystidium, and hence, in view of the spore characters, well developed veil, and the non-darkening stipe, place this collection within the *G. cerina* complex. The color of the pileus approaches that of *G. turfosa*.

7. **Galerina luteolosperma** sp. nov.

Pileo 8–10 mm lato, conico, glabro, udo, hygrophano, sordide pallide fulvo; lamellis confertis, latis, pallide fulvis; stipite 4–5 cm longo, 1 mm crasso, fragili, ochraceo, leniter fibrilloso; sporis 8–11 × 5–5.5 μ, pallide luteolis (in KOH); cheilocystidiis 28–36 × 8–11 μ, ventricoso-capitatis; Smith n. *43722*.

Pileus 8–10 mm broad, conic with a straight margin, becoming conic-campanulate, surface glabrous, moist, hygrophanous, color an even dull ochraceous tawny when moist, dingy tan faded and near cinnamon brown dried, conspicuously striate when moist; flesh thin, fragile, odor and taste not recorded; lamellae close, moderately broad, hooked, ascending, about ochraceous tawny; stipe 4.5 cm long, about 1 mm thick, equal, fragile, "pinkish buff" over all, with pallid fibrils from veil over lower half at first, apex pruinose.

Spores 8–11 × 5–5.5 μ, slightly inequilateral in side view, ovate in face view, *pale yellow* when first revived in KOH but only gradually becoming ochraceous tawny, smooth or nearly so and plage area not distinctly delimited or a few spores showing a faint ragged boundary line; basidia 4-spored; pleurocystidia none; cheilocystidia 28–36 × 8–11 μ, ventricose-capitate as in *G. heterocystis;* hyphae of cap trama with heavy incrustations; clamp connections present but not abundant.

Habit, habitat and distribution. Gregarious on *Sphagnum,* Trout Lake, Mich., Oct. 4, 1953.

Material examined. Sm-*43722,* TYPE.

Observations. This species differs from *G. sphagnorum* in the capitate cheilocystidia and from *G. tibiicystis* in the presence of a veil and nearly smooth spores. The heads of the cheilocystidia are about 5–8 μ in diam. and the necks about 3 μ.

8. **Galerina cascadensis** sp. nov.

Pileo 12–18 mm lato, glabro, obscure ochraceo-brunneo; lamellis confertis, obscure ochraceo-brunneis; stipite 40–45 × 1.5–2.5 mm, melleo ad apicem, obscure ochraceo-brunneo ad basin, veli vestigiis fibrilloso; sporis 9–11(–12) × 5–6 μ, subtiliter ruguloso-verruculosis, disco suprahilari levi praeditis; cheilocystidiis ventricoso-capitatis vel cylindraceo-subcapitatis, 40–70 × 6–12 μ; hyphis fibulatis; ad quisquilias detriti nive delapsi, Mt. Rainier National Park, Washington, U. S. A.; Smith n. *41144.*

Pileus 12–18 mm broad, obtusely conic becoming conic-umbonate with a spreading margin, glabrous, moist, hygrophanous, dark ochraceous tawny and faintly striate moist, cinnamon-buff faded; odor and taste not recorded; lamellae close, narrow, adnate, dark ochraceous tawny, edges whitish and fimbriate; stipe 40–45 mm long, 1.5–2.5 mm thick, somewhat enlarged downward, honey-color above, dark ochraceous tawny below, lower half with scattered fibrils from the rudimentary veil, apex pruinose.

Spores 9–11(–12.5) × 5–6(–7) μ, somewhat inequilateral in side view, ovate in face view, outer wall faintly rugulose-warty, suprahilar area smooth and marked off by a ragged line, ochraceous tawny in KOH; basidia 2-, and 4-spored; pleurocystidia none; cheilocystidia abundant,

40–70 × 6–12 μ, ventricose-capitate to cylindric-capitate or some with long narrow necks and subacute apices and a narrowly ventricose base, capitate individuals at times with a prolongation from the capitulum which in turn may or may not be capitate; gill trama regular; pileus trama homogeneous; clamp connections present.

Habit, habitat and distribution. Subcespitose on debris of an old avalanche, Green Lake, Mt. Rainier National Park, Wash., Oct. 19, 1952.

Material examined. Sm-*41144*, TYPE.

Observations. The stature of the carpophore reminds one of *Cortinarius acutus* and the combination of large, typically *Galerina* spores and long cheilocystidia of the *G. tibiicystis* type is distinctive microscopically. In *G. cortinarioides* the spores are calyptrate and the cheilocystidia are the usual fusoid-ventricose type. *G. casadensis* differs from *G. uncialis* in stipe characters and habitat as well as in the size of the cheilocystidia.

9. **Galerina subbadia** sp. nov.

Pileo 5–10 mm lato, applanato-umbonato, margine incurvo, glabro, rutilo-brunneo vel cinnamomeo-brunneo; lamellis adnatis ochraceo-brunneis; stipite plus minusve 10 mm longo, 0.7 mm lato, curvato, admodum fragili, velo haud viso; sporis 7–8 × 4–4.5 μ, asperulis, disco suprahilari levi praeditis; pleurocystidiis subnullis; cheilocystidiis 28–36(–44) × 5–8 μ, cylindraceo-capitatis vel fusiformi-ventricosis apice angustissimis; hyphis fibulatis; ad lignum putridum abietinum, Blue River, Oregon. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *7845*.

Pileus 5–10 mm broad, plane, with a low obtuse umbo, margin incurved, surface glabrous, color "russet" to "cinnamon brown" and translucent striate moist, hygrophanous and fading to "ochraceous buff"; flesh thin, membranous, fragile, odor and taste not distinctive; lamellae moderately close and broad, bluntly adnate, ochraceous tawny, edges somewhat crenate; stipe about 10 mm long and 0.7 mm thick, curved, equal or base slightly enlarged, very fragile, color of cap or paler; no veil seen.

Spores 7–8 × 4–4.5 μ, inequilateral in side view, ovate in face view, tawny or darker in KOH, exospore wrinkled causing surface to appear obscurely roughened, suprahilar depression distinctly marked off and smooth, with an apical callus; basidia 4-spored, hyaline in KOH; pleurocystidia rare to absent, similar to cheilocystidia if present; cheilocystidia 28–36(–44) × 5–8 μ, some cylindric-capitate, others fusoid-ventricose with very narrow neck (2–3 μ), all hyaline and thin-walled in KOH or only the basal part pale ochraceous in KOH; gill trama subparallel,

ochraceous tawny in KOH from heavy incrustations of pigment, pilocystidia similar to cheilocystidia, scattered; clamp connections present.

Habit, habitat and distribution. Gregarious on a fir log, Blue River, Oregon, Oct. 16, 1937; Sm-*7845*. It was found in Michigan June 25, 1951 on a conifer log at Cut River Bridge, U. S. highway No. 2.

Material examined. Sm-*7845*, TYPE; Sm-*36419*.

Observations. This is one of the convex to obtuse species of the stirps *G. triscopa*. The pilocystidia were present and similar in both collections, but the spores in *36419* have a tendency to be narrower in relation to their length. The majority of the cheilocystidia are capitate to subcapitate. The pleurocystidia noted in the type were probably at injured places in the hymenium and are not of taxonomic importance.

This species differs from *G. laticeps* in the narrower, less ornamented spores and presence of pilocystidia as well as in the darker colored pileus.

10. **Galerina vinaceobrunnea** sp. nov.

A *G. stylifera* colore pilei vinaceo-brunneo differt. Ad lignum coniferarum. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *34149*.

Pileus 10–30 mm broad, obtuse to convex with an incurved margin, expanding to nearly plane or with an inconspicuous umbo, surface glabrous, subviscid, "Roods brown" to vinaceous tawny and retaining a dull cinnamon cast when faded; flesh thin, odor and taste not recorded; lamellae moderately broad, adnate, nearly horizontal, dull brownish becoming ochraceous tawny, edges even; stipes 30–50 mm long, 1.5–3 mm thick at apex, somewhat enlarged downward, surface thinly fibrillose over lower part from veil remnants, apex fibrillose-punctate, rusty brown below, paler above and darkening slightly from the base up in age.

Spores 6–7 × 4 μ, ellipsoid to subovoid, *smooth*, ochraceous tawny in KOH; basidia 18–22 × 6–7 μ, 4-spored, hyaline in KOH; pleurocystidia none; cheilocystidia ventricose-subcapitate, 18–26 × 3–8 μ, the head 3.5–4 μ, thin-walled, hyaline in KOH; gill trama interwoven, pale tawny in KOH; pileus with a distinct hyaline gelatinous pellicle, beneath this the tramal body is tawny or paler and of interwoven hyphae, pigment encrusted; clamp connections present.

Habit, habitat and distribution. Gregarious to subcespitose on conifer wood, Miller's Bog, Burt Lake, Mich., Sept. 23, 1949.

Material examined. Sm-*34149*, TYPE.

Observations. The important difference between this species and *G. stylifera* is one of color, and in addition the stipe does not darken as much.

11. **Galerina agloea** sp. nov.

Pileus 8–15(–25) mm lato, ochraceo-brunneo, glabro; lamellis confertis, carneo-alutaceis dein ochracea-brunneis; stipite 40–60 × 1–2 mm, haud fragili, biso ad basin, veli vestigiis tenuibus, grisellis induto; sporis 7–8.5 × 4–4.5 μ, levibus; pleurocystidiis nullis; cheilocystidiis fusiforme-ventricosis, apice attenuatis, subacutis, 26–40 × 5–9 μ; epicute pilei haud bene evoluta, haud gelatinosa; hyphis fibuligeris; ad acus coniferarum. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *40462*.

Pileus 8–15(–25) mm broad, obtuse with a bent-in margin young, convex to nearly plane in age, at times with a slight umbo, surface glabrous, moist, hygrophanous, tawny to ochraceous tawny moist, near pinkish buff when faded, striate when moist; flesh thin, odor and taste not recorded; lamellae close, narrow to moderately broad, adnate, ochraceous tawny mature, when young near pinkish buff, edges even; stipe 40–60 mm long, 1–2 mm thick, equal, not markedly fragile, darkening to bister starting at the base and progressing upward, apex dingy honey color, veil slight and leaving thin coating of grayish fibrils over the lower portion.

Spores 7–8.5 × 4–4.5 μ, ovate in face view, obscurely inequilateral in side view, pale ochraceous tawny in KOH, smooth; basidia 4-spored; pleurocystidia none; cheilocystidia fusoid-ventricose with subacute apices, rarely somewhat capitate, 26–40 × 5–9 μ, hyaline in KOH; gill trama somewhat interwoven, pale cinnamon to pallid revived in KOH, subhymenium thin and not distinctive; pileus trama homogeneous, dull cinnamon to pallid in KOH, no appreciably differentiated pellicle; clamp connections present.

Habit, habitat and distribution. Gregarious on conifer needles, Carbon River, Mt. Rainier National Park, Wash., October 2, 1952.

Material examined. Sm-*40462*, TYPE; Sm-*31612*; *41003*.

Observations. The incurved margin of the pileus and darkening stipe separate this species from *G. fuscobrunnea*. In addition there is a slight difference in spore size. *G. agloea* can easily be passed by in the field for *G. stylifera* but the caps of both it and *G. mammilata* are viscid.

12. **Galerina jaapii** sp. nov.

Pileo 6–18 mm lato vel latiore, cinnamomeo-brunneo; lamellis latis, subdistantibus, cinnamomeo-alutaceis, dein brunnescentibus; stipite 30–100 mm longo, 1–2.5 mm crasso, annulato; pleurocystidiis nullis; sporis 10–13 × 6–7 μ, marmoratis; hyphis fibulatis; basidiis bi- vel tetrasporis; ad muscos in Germania, Jaap, sub nomine *Pholiota mycenoidi*, typus (Fungi Selecti Exsiccati 10, *VIII*, 1903).

Pileus 6–18 mm broad, broadly conic, expanding to plano-umbonate, rarely mammillate, surface glabrous, lubricous, nearly "cinnamon-brown"

on disc or over all when young, soon dull tawny to ochraceous tawny over the margin, conspicuously translucent-striate, hygrophanous, fading on disc first to pinkish buff; flesh very soft and watery, odor and taste none; lamellae broad, subdistant, horizontal, bluntly adnate, cinnamon buff young, finally dark ochraceous tawny, edges even; stipe 30–100 mm long, 1–2.5 mm thick at apex, equal or slightly larger downward, surface glabrous, naked and moist except for a thin white submembranous annulus, honey-color near the apex or over all at first, ochraceous tawny over lower half in age.

Spores (9–)10–13 × 6–7 μ, narrowly inequilateral in side view, elongate-ovate in face view, minutely roughened over all except the smooth suprahilar area; basidia 2-spored; pleurocystidia none; cheilocystidia 36–54 × 8–11 μ, somewhat ventricose below with a neck 4–7 μ thick and an obtuse to subcapitate apex (5–10 μ thick), hyaline but often with refractive granular material in the apex (revived in KOH); no pilocystidia seen; clamp connections present.

Habit, habitat and distribution. On moss, scattered to gregarious in small bogs or along the margins of swamps, summer and fall. It is known from Europe (France and Germany) and North America (Pacific Northwest and Great Lakes Region).

Material examined. Jaap, Fungi Sel. Exsic, 10, *VIII*, 1903, TYPE; Sm-*42568*, Pike Lake, Luce Bounty, Mich.; also *4118; 24497;* Mains *5114*.

Observations. The above description is entirely based on an unusually abundant fruiting of the species in a small pothole bog near Pike Lake (Sm-*42568*). The description given by Kühner on the basis of his own collections in France and Jaap's exsiccatum is very detailed and permits a complete comparison with our North American specimens. The spores are slightly larger, and so are the maximum measurements of pileus and stipe. The cheilocystidia also reach larger dimensions, but there is no doubt in our minds regarding the identity of both the American and European collections.

However, Kühner calls his species *Galerina mycenoides*, transferring the Friesian *Agaricus mycenoides* to *Galerina*. Before Kühner (**1935**) published his new interpretation of *A. mycenoides* Fr. this species was well established in the literature in the sense of Boudier (**1905–1910**) and later Overholts (**1928**). The concept of Overholts clearly described a fungus of the *Conocybe* type, i.e. with truncate spores. In the character of the annulus this concept is also in perfect agreement with the Friesian descriptions ("annulo integro"). It was unquestionably the well-formed entire membranous annulus which led Fries to place the species in *Pholiota*. Since there can be only one *A. mycenoides* Fries

we take the position that a well established earlier concept has the priority.

GALERINA JAAPII f. **mamillata** (Sing.) comb. nov.

Galerina mycenoides f. *mamillata* Singer, Act. Inst. Bot. Kom. II, Pl. Crypt. **6**: 480. 1950.

Pileus 3–15 mm broad, obtusely conic when young, becoming plane, with an abrupt conic umbo, some caps remaining conic-campanulate, moist, glabrous, hygrophanous, "ochraceous tawny" over disc and striae, paler tawny between them, transparently striate to the disc, fading to pale buff and becoming almost smooth to slightly sulculate when faded; flesh very thin and watery, soft, odor none; taste perfectly mild; lamellae distant to subdistant (12–15 reach the stipe), with one tier of lamellulae, broadly adnate, moderately broad (1–3 mm), with even edges, concolorous with the pileus or paler, finally ocher brown; stipe 20–30 mm long, 1–1.5 mm thick, equal, tubular, soft and fragile but snapping when young fresh stipes are broken, concolorous with pileus or paler, over lower portion, dingy light ochraceous at apex, sometimes unicolorous, glabrous execpt for the apical zone or annulus; annulus both white and fibrillose, at times evanescent, smooth, at first distant.

Spores 8–11 × 4–5 μ in 4-spored caps, and (8–)10–16(–20) × 5.5–8(–8.5) μ in 1–2-spored caps, obscurely inequilateral in side view, narrowly ovate in face view, tawny in KOH, with slightly rugulose exosporial ornamentation, with an apparently smooth but poorly marked plage, with apical callus or a tiny germ pore; basidia 25–33 × 6.5–8.5 μ, some caps 4-spored, some 2-spored, some very irregular (1–2–4-spored); sterigmata in 2-spored form 6.5–8.5 μ, pleurocystidia none; cheilocystidia abundant, 28–45(–65) × 8–14 μ, fusoid-ventricose below with attenuate or cylindric obtuse apex or with constriction to 2.5–4 μ in diam. underneath a subcapitate tip, capitulum 5–9.5 μ in diam., thin-walled, mostly hyaline but occasionally a few with smoky yellow content when revived in KOH; pileus trama homogeneous, pale ochraceous toward the surface; no pellicle differentiataed; all hyphae with clamp connections.

Habit, habitat and distribution. Gregarious on wet soil, along ditches and often among mosses such as *Bryum* and *Nelodium*. Known from Karelia, Tataria (U.S.S.R.) and Washington in North America. Very likely the variety has a wider distribution in Northern Europe and Northwestern North America, but it is apparently not common.

Material examined. Singer & Freindling from near Kivatch, Karelia, Sept. 1936 (LE)-TYPE; Vasilieva from near Kazan, Tataria (LE); Smith-*16302*, Baker Lake, Washington, August 22, 1941 (MICH).

Observations. This form is interesting not so much because of its

papilla as because of the fact that in Washington 4-spored specimens were found almost side by side with (1–)2-spored carpophores.

13. **Galerina decipiens** sp. nov.

Pileo 5–10 mm lato, obtuse conico, margine stricto, ochraceo-brunneo vel melleo; odore nullo; lamellis adnatis dente decurrente, pallide alutaceis, dein brunnescentibus; stipite 10–30 mm longo, 0.7–1.2 mm crasso; velo fugacissimo, hyalino, sericeo, in maturis nullo; sporis 7–9(–10) × 4.5–5.5 μ, punctato-asperis, disco suprahilari levi bene evoluto; pleurocystidiis nullis, cheilocystidiis ampullaceis, multis capitatis vel subcapitatis ad apicem, 32–44 × 7–10.8 μ, hyphis fibuligeris; ad *Hypnum* et *Heterophyllium* supra terram lignaque putridissima. Specimen typicum in Herb. Chic. Mus. Nat. Hist.; Singer n. 91.

Pileus (3–)5–10(–12) mm broad, 3–7 mm high, very variable in shape, acutely to obtusely conic with a straight margin, later campanulate with or without a small and often acute papilla, glabrous, sometimes slightly sulcate, moist and hygrophanous, pale fulvous ("ochraceous tawny" or *Peruvian brown*), margin paler at times, usually paler between the striae, transparently striate up to three-quarters of the radius, fading to buff or pale pinkish buff and smooth when dry; fresh thin and only moderately fragile, odor none; taste mild; lamellae narrow to broad, ascending, adnate with a tooth, rounded-adnexed, adnate, at times seceding, moderately close to distant, pale buff when young, near tawny at maturity, edges even; stipe 10–30 mm long, 0.7–1.2 mm thick, reaching 1.2–2 mm at base, nearly equal, pruinose above, glabrous below (veil absent in mature carpophores), pallid ochroleucous or stramineous pallid to pale sordid cinnamon (*Pablo*) throughout and not darkening appreciably from the base upward in age but sometimems in the middle or below with brown (*Russian calf* or *hazel*) stains where handled; veil consisting of faintly marked silky hyaline fibrils which are soon evanescent leaving the lower part of the stipe perfectly glabrous.

Spores (7–)8–10(–11) × 4.5–5.5 μ, inequilateral in side view, ovate in front view, tawny to ochraceous tawny in KOH, with a smooth plage and a wrinkled, distinctly punctate-rough to verrucose exosporial ornamentation covering the remainder, at times this layer showing a slight tendency to separate forming blisters, but these not confined to the plage-border area; basidia 21 × 6.8–7.5 μ, 4-spored; pleurocystidia none; cheilocystidia abundant, fusoid-ventricose below, ampullaceous with obtuse tips, a majority subcapitate to capitate, 32–44 × 6.8–10(–11) μ, neck usually 3–5.3 μ thick and often flexuous, capitulum 4.3–6.8 μ in diam., wall thin, hyaline, rarely yellowish in the ventricose part in KOH; hyphae of pileus trama rather heavily incrusted with tawny to yellowish pigment; epicutis poorly developed, not gelatinized, without pilocystidia; caulocystidia similar to cheilocystidia and present mostly at apex of stipe; all hyphae with clamp connections.

Habit, habitat and distribution. Solitary to gregarious on moss (so far never on *Polytrichum* or *Sphagnum*) over soil or rotten wood, not uncommon during the summer (June-September) in northern Michigan.

Material examined. Singer N-*91*; N-*431*, TYPE; N-*1277*; N-*1277a*; N-*1278a*; N-*1278b*; N-*1279c*; (F). Sm-*41444*; *41516*; *41527*; *41666*; *41875*; *41876*; *41897*; *41899*; *42000*; *42009*; *42155*; *42165*; *42166*; *42171*; *42172*; *42173*; *42178*; *42179*; *42196*; *42199*; *42268*; *42271*; *42272*; *42273*; *42587*; *42590*; *42591*; *42672*; *42906*; *43033*; *43013*; *44024*.

Observations. *G. hypnorum* is, perhaps, the most closely related species but differs in the consistently larger spores which are much less ornamented and in more of the cheilocystidia being non-capitate. Our abundant collections have established beyond the shadow of a doubt that *G. decipiens* is a constant and readily recognizable species in the Great Lakes Region agaric flora. Although we have encountered a number of variations, these have not shown an intergradation with *G. hypnorum*. A number of the variations are known from single collections and because of the small number of carpophores involved along with the nature of the characters, we do not care to give them formal designations at this time. They are, however, worth putting on record.

Sm-*41666*. Spores 7–8.5 × 4.5–5 μ; stipe pale yellow over all and no veil present in buttons. It grew on moss over sandy soil, Tahquaenon Falls State Park, July 23, 1953.

Singer N-*537*. Pileus 6–10 mm broad, soon conic-applanate to convex with a broad obtuse umbo, color rather dark (*tortoise* tinged *gypsy*, umbo *cowboy* or lighter), with a tendency to become deep badious around margin in drying out, when moist transparently striate over three-fourths of the radius and only slightly paler between striae; odor none, taste mild; lamellae horizontal, distant, ventricose, rounded-adnate, 2.2 mm broad, ochraceous brownish; stipe 23–25 mm long, 0.8–1.2 mm thick, pale dingy cinnamon buff (*pl 12– F 6*), eventually with a tendency to become deeper colored (*Russian calf*) over midportion, base subbulbose, apex pruinose, with slight silky fibrils from the veil.

Spores 8.3–9.8 × 5.3–5.5 μ (12 × 5.7), strongly pigmented, with well-developed, adherent, exosporial ornamentation; basidia 21 × 6.8 μ, 4-spored; cheilocystidia mostly subcapitate, 24–42 × 5–9 μ, minority ampullaceous, neck 3–5.3 μ thick, capitulum 4.5–6.8 μ broad; cuticle of pileus poorly developed, hyaline, thin, not gelatinous, without pilocystidia; clamps present. Collected on Ferry Island, Douglas Lake, Mich., Che-

boygan County, on rotten trunk covered by *Heterophyllium haldanianum* and other hypnaceous mosses.

This collection has many of the characters of *G. rugisperma*, only the exosporium does not loosen and that species, so far as is known, is western. If the color of the spores and the adherent exosporium of Singer's *N-537* are found to be constant, the variant should be given formal recognition as a taxon. It should also be pointed out that the trend of variation noted here is not toward the characters of *G. hypnorum*.

Galerina decipiens var. **separans** var. nov.

A *G. decipienti* var. *decipienti* exosporio separante differt. Ad ligna putrida muscosa. Specimen typicum in Herb. Univ. Mich. conservatum, Smith n. *41739*.

Pileus 6–10 mm broad, obtusely conic with a straight margin, glabrous, moist, hygrophanous, dark ochraceous tawny over the disc, paler on margin, transulcent striate when moist, fading to pinkish buff or paler; flesh very thin, odor and taste not distinctive. Lamellae subdistant to close, ascending adnate, moderately broad, ochraceous tawny. Stipe 10–25 mm long, 0.7–1 mm thick, equal, glabrous, naked, dingy honey color to watery ochraceous above, darker ochraceous tawny below.

Spores 8.5–11 × 5–5.5 μ, inequilateral in side view, narrowly ovate in face view, ochraceous tawny or a little darker in KOH, wrinkled-warty over all except for the smooth plage, exosporium separating around the edge of the plage forming loose flaps or small blisters and also separating slightly in various places over the spore surface; basidia 4-spored; pleurocystidia none; cheilocystidia 30–46 × 7–11 μ, slightly ventricose below and with an elongated neck and obtuse to capitate apex, hyaline, smooth, thin-walled; no pilocystidia seen; clamp connections present.

Habit, habitat and distribution. Gregarious on mossy logs, Wilderness Park, Emmet County, Mich., July 29, 1953.

Material studied. Sm-*41739*, TYPE; *41847*; *41883*; *41900*; *42007*; *42016*; *42042*; *42180*; *42197*; *42198*.

Observations. The spores of *G. rugisperma* are darker in KOH and the exosporium envelops the spore loosely. That species has, in addition, a more strongly developed veil and horizontal gills. The lack of a veil, slightly larger spores, and the readily recognizable tendency for blister formation on the spores distinguish var. *separans* from the type variety. We have observed considerable intergradation between this and the type variety, however, in spore characters and in the presence of a very thin veil. In view of this, it hardly seemed justifiable to place the type of var. *separans* in *Calyptrospora* where it would have to

be recognized as a species. We have found both varieties in the same habitats and at the same time of year.

G. hypnorum differs in having larger, smoother spores in which loosening of the episporum is lacking or very slight.

14. **Galerina emmetensis** sp. nov.

Pileo 2–11 mm lato, ochraceo-brunneolo vel cinnamomeo-brunneo, mediocriter striatulo; lamellis alutaceo-flavis dein pallide cinnamomeo-brunneis, adnexis vel adnatis; stipite 13–25 × 0.8–1.2 mm, sordide melleo, basi haud decolorante, velo sericeo fugaci; sporis 8–9 × 4.5–5.2 μ, subtiliter marmoratis vel subpunctatis, interdum mucronatis, disco suprahilari levi praesente; pleurocystidiis nullis; cheilocystidiis ampullaceis, apice cylindraceis vel subcapitatis 28–36 × 6–12 μ; hyphis fubulatis; ad *Bryum Sphagnumque* supra humum acusque putridas locis humidis, Michigan, U.S.A. Specimen typicum in Herb. Chic. Mus. Nat. Hist. and Herb. Univ. of Mich. conservatum, Singer n. *83*.

Pileus 2–11 mm broad, obtusely conic to campanulate to nearly convex, glabrous, moist and hygrophanous, dull tawny to pale cinnamon brown (*Saratoga*, disc and striae *raw sienna*), only moderately striate when moist, fading to near cinnamon buff or light ochraceous; flesh thin, odor none, taste mild to farinaceous; lamellae buff yellow (*spruce y*), pale cinnamon-brown at maturity with fimbriate edges in many carpophores, broad, ventricose, ascending to nearly horizontal, medium close to distant, adnexed or adnate; stipe 13–25 mm long, 0.8–1.2 mm thick, equal, or with a slightly thickened base, dingy melleous over all (*pl. 11 H 7*) but not darkening at the base, slightly pruinate at the apex, with a faint yellowish pallid silky veil which soon disappears entirely.

Spores (7–)8–9(–10.2) × (3.5–)4.5–5.2(–5.5) μ, smooth to faintly marbled-subpunctate, slightly inequilateral in side view, ovate and some mucronate at face view, with faintly bounded plage and smooth in the plage area, with apical callus; basidia 40–24 × 6.7–7.5, 4-spored, or rarely varying 1-, 2-, 3-spored; pleurocystidia none; cheilocystidia 28–36(–44) × 6–12 μ, ventricose at base, neck elongated, often flexuous, and usually 3–5 μ thick but sometimes at apex nearly as broad as in the ventricose (subclavate) part, i.e., apex cylindrical to subcapitate, hyaline when fresh but often with yellow contents when revived in KOH; caulocystidia similar to cheilocystidia (some vesiculose cells present in both areas); tramal hyphae strongly pigment-incrusted; pilocystidia lacking; clamp connections present.

Habit, habitat and distribution. Gregarious on *Bryum* and *Sphagnum* over humus and rotten needles, in wet places (around dried up pools in a bog), between Brutus and Pellston, Mich., Emmet County, Michigan, June–July, 1953.

Material examined. Singer *N-83* (MICH, F), TYPE; Sm-*41245; 41420; 41421; 41515.*

Observations. This differs from *G. hypnorum* in decidedly smaller spores and from *G. decipiens* in color and more faintly ornamented spores. In Sm-*414515* taste was farinaceous and the spores (very rarely) were seen to have one or more small blisters. This would seem to indicate a relationship to the *Calyptrospora* group.

GALERINA EMMETENSIS var. **intermedia** var. nov.

Pileus 5–10 mm latus, castaneus vel subcastaneus demum fulvus vel pallide fulvus; sapor farinaceus; lamellae subochraceae, latae, confertae; stipes 10–20 mm longus, 1–1.5 mm crassus, sordide brunneus, deorsum haud decolorans; sporae 7–9 × 4–4.5 μ; cheilocystidia 30–42 × 7–10 μ, fusoideo-ventricosa. Specimen typicum in Herb. Univ. of Mich. conservatum; lectum prope Brutus, Mich., July 6, 1953; Smith n. *41515.*

Pelius 5–10 mm broad, obtuse to convex, margin straight at first, glabrous except for faint fibrils along the margin in young caps, color "Hay's russet," "auburn" to "ochraceous tawny" (*Alamo* to *hazel*), transparently striate finally over three-fourths the radius, hygrophanous, paler when faded; flesh thin, fragile, odor none, taste farinaceous; lamellae pallid ochraceous becoming horizontal and adnexed, edges pubescent under a lens; stipe 10–20 mm long, 1–1.5 mm thick, slightly attenuated upward or equal, fragile, pruinose above, not distinctly discoloring at base in age, stramineous-melleous over all and in age dingy ochraceous to brownish (reaching *hazel*); veil rudimentary, silky, white, appressed, rarely forming an indistinct apical zone.

Spores (6–)7–9 × (3.5–)4–4.5 μ, slightly inequilateral in side view, ovate in face view, smooth to faintly marbled but with a faint line bordering the plage area and occasionally with one or more small blisters (separations) over the remainder, dark ochraceous tawny in KOH; basidia 14–17 × 6–8 μ, 4-spored; pleurocystidia absent; cheilocystidia (22.5–)30–42(–56) × 7–10 μ, narrowly fusoid-ventricose to subclavate and then usually with flexuous walls or constrictions, some subcapitate, hyaline in KOH; pileus trama with pigment-incrusted hyphae; clamp connections present.

Habit, habitat and distribution. Scattered among sedges and wet moss in a recently dried up bog pool in a *Polytrichum* bog, near Brutus, Mich., July and August.

Material examined. Singer *N-1248; N-1205;* Sm-*41515,* TYPE.

Observations. *G. aberrans* appears close to this variety but has a truly darkening stipe and habitat on burned moss, and it faded to a vinaceous brown instead of the usually buff color of most galerinas, but is quite

similar in the initial cap color. The taste of *G. aberrans* was not recorded.

15. **Galerina aberrans** sp. nov.

Pileo 5–10 mm lato, obtuse conico dein expanso, castaneo-brunneo vel castaneo, hygrophano, in siccis vinaceo-brunneo; lamellis cum pileo concoloribus; stipite circiter 20 × 1.5 mm, veli vestigiis appresse fibrilloso, sordide ochraceo ad apicem, umbrino ad basin; sporis 7–8 × 3.5–4 μ, subtilissime marmoratis; disco suprahilari levi praesente; pleurocystidiis nullis; cheilocystidiis subcylindraceis vel subventricosis et ad apices flexuosis attenuatis obtusisque; hyphis fibulatis; ad *Polytricha* loco subusto, Michigan, U.S.A.

Pileus 5–10 mm broad, obtusely conic young, expanding to obtusely umbonate or convex, surface moist and hygrophanous, glabrous, "chestnut brown" to "auburn" (bay), fading to vinaceous brown ("Pecan brown"), striate moist, opaque faded; flesh thin, fragile, concolorous with surface, odor and taste not recorded; lamellae close to subdistant, adnate, seceding, color of pileus, edges white-floccose; stipe ± 20 mm long, 1.5 mm thick, enlarged above and narrowed at base, pruinose above, with scattered appressed fibrils from the remains of the rudimentary veil, sordid ochraceous above, darker (umber brown) below.

Spores 7–8(–9) × 3.5–4(10.5 × 5) μ, very slightly inequilateral to subovate in side view, subelliptic to narrowly ovate in face view, plage with a distinct boundary, remainder of surface faintly marbled to smooth, cinnamon tan revived in KOH, apical pore not apparent under oil; basidia 18–20 × 5–6 μ, subcylindric, 4-spored and 2-spored; pleurocystidia none; cheilocystidia 26–34(–40) × 6–12 μ, abundant, subcylindric to subventricose with flexuous necks and obtuse apex, hyaline, thin-walled and smooth (in KOH); gill trama of interwoven hyphae with brown incrusting pigment (in KOH); pileus trama homogeneous, the cells near the surface with dark rusty brown incrusting pigment; clamp connections present.

Habit, habitat and distribution. Gregarious on *Polytrichum* in a burned area, Mud Lake Bog, Whitmore Lake, Mich., Sept. 25, 1933.

Material examined. Sm-*33-1024*, TYPE.

Observations. Although the spores are practically smooth and small and the cheilocystidia fairly narrow, the fungus does not belong in the *G. sideroides* stirps. The rich dark colors, nearly smooth inequilateral spores with a definite suprahilar depression, fusoid-ventricose, obtuse cheilocystidia, and habitat are a distinctive combination of characters. It is close to the stirps *paludosa* but lacks the veil development of that group.

16. **Galerina vexans** sp. nov.

Pileo 10–15 mm lato, margine stricto in juvenilibus, obtuse conico, dein obtuse campanulato vel plano umbonatoque, primum zona velari subtili marginali praedito, mox glabrescente, nitido lubricoque, pallide flavido vel ochraceo, demum in disco brunnescente, conspicue striato; lamellis carneo-alutaceis dein pallide ochraceo-brunneis, adnatis, subdistantibus; stipite 40–60 mm longo, 1.5–2.5 mm crasso, aquose ochraceo, ad apicem pallide melleo, ad basin hyalino-albido; velo fibrilloso, sparso, flavido-pallido; sporis 9–11 × 5–6 μ, levibus, disco suprahilari levi haud delimitato; pleurocystidiis nullis; cheilocystidiis 36–58 × 8–12 μ, ampullaceis; epicute haud gelatinascente; hyphis fibulatis. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *42824*.

Pileus 10–15 mm broad, obtusely conic, the margin straight when young, expanding to obtusely campanulate or plano-umbonate, at first with a faint zone of veil fibrils along the margin, soon glabrous, shining and lubricous when wet (buttons slightly viscid), color pale yellow to ochraceous ("antimony yellow" to near "ocher yellow") over-all when young, disc finally becoming darker (near "buckthorn brown"), conspicuously translucent-striate; flesh thin, dull yellow, odor and taste mild; lamellae pale pinkish buff becoming pale ochraceous tawny, adnate, moderately broad and becoming ventricose, subdistant; stipe 40–60 mm long, 1.5–2.5 mm thick, equal or thickened slightly downward, base hyaline whitish, middle portion watery ochraceous, apex pallid honey color, surface fibrillose from remains of a thin yellowish-pallid veil, apex pruinose.

Spores 9–11(–12) × 5–6 μ, smooth (no plage line seen under highest magnification), dark rusty brown in Melzer's reagent, fulvous in KOH; basidia 4-spored; pleurocystidia none; cheilocystidia abundant, 36–58 × 8–12 μ, slightly ventricose at base and with an elongated flexuous neck and obtuse apex, smooth, hyaline, thin-walled; cuticle poorly differentiated (not gelatinous), all hyphae of pileus trama with incrusted pigment or yellowish walls; clamp connections present.

Habit, habitat and distribution. Gregarious on moss in open but shaded areas, Pt. Aux Chenes and Wilderness Park, Mich.

Material examined. Sm-*42824*, TYPE; *42825*; *42963*; *42964*; *43005*; *43008*; *43472*; *43495*; *43715*; *43717*; *43719*; *43844*; *43921*; *44035*.

Observations. This species differs from *G. lacustris* in having smooth cheilocystidia mostly with no apical enlargement, spores which lack a plage line, and a truly yellow pileus when young. The yellow of the veil is distinct in good material but is very pale and easily overlooked. In the field *G. mycenopsis* is readily distinguished by its dingy colors. Dried specimens are readily distinct from *G. vexans* by their much paler spores both in Melzer's reagent and KOH. Also, *G. mycenopsis* is characteristic of cold, montane habitats and seems to fruit

best after light frosts. Actually *G. vexans* is difficult to distinguish, in the field, from *G. heterocystis*. At Wilderness Park in 1953 it was found impossible to distinguish them at sight. They are, of course, very distinct under the microscope.

17. **Galerina naucorioides** sp. nov.

Pileo 8–15 mm lato, convexo vel plano, subfibrilloso in juvenilibus, glabrescente, obscure castaneo-brunneo; lamellis angustis, distantibus, adnatis, pileo concoloribus; stipite 10–20 × 1–2 mm, pileo concolori, sparse fibrilloso ad basin; sporis 10–12(–13) × 5–6 μ, levibus vel subtiliter rugulosis, disco suprahilari bene delimitato praeditis; pleurocystidiis typicis nullis, sed cystidiolis (?) clavatis angustis brunneolis praesentibus; cheilocystidiis 32–44 × 7–11 μ, fusiformi-ventricosis, apice angustatis, flexuosis obtusis; hyphis fibulatis. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *33-995*.

Pileus 8–15 mm broad, convex to plane or slightly umbonate, margin incurved when young, slightly fibrillose young, glabrescent, moist and hygrophanous, dark brown (near "russet") fading to pale tan; lamellae narrow, distant, adnate, concolorous with pileus, edges even; stipe 10–20 mm long, 1–2 mm thick, contorted to curved, narrowed at base, enlarged at apex, concolorous with pileus, pruinose above, sparsely fibrillose toward the base.

Spores 10–12(–13) × 5–6 μ, narrowly subinequilateral in side view, narrowly ovate in face view, ochraceous tawny in KOH, *smooth* or slightly rugulose, plage marked by a ragged line, with an apical callus; basidia 30–32 × 8–9 μ, hyaline in KOH, 4-spored; pleurocystidia present on or near edges, 32–44 × 7–11 μ, fusoid-ventricose with obtuse apices and flexuous necks, hyaline and thin-walled in KOH; gill trama subparallel to interwoven, yellow-brown in KOH from incrusting pigment; pileus trama homogeneous, darker yellow brown in KOH than gill trama; clamp connections present.

Habit, habitat and distribution. Single to gregarious on debris and sticks on moist soil, Huron Mts., Mich., September.

Material studied. Sm-*33-995*, TYPE.

Observations. This is a *Naucoria*-like species with the cap-margin incurved, a dark brown pileus, and nearly smooth, narrow, non-calyptrate spores. *G. carbonicola* appears to be closely related in its incurved cap-margin and dark colors but differs in habitat and smaller spores which are more conspicuously roughened.

18. **Galerina tundrae** sp. nov.

Pileo 10–20 mm lato, ochraceo-castaneo vel brunneo, opaco, lamellis distantibus vel subdistantibus, latis, ochraceo-brunneis; stipite 10–20 mm longo, C. 2 mm lato, ad apicem cum lamellis ad basin cum pileo concolori, veli reliquiis pallidis sparsis

induto; sporis 10–13 × 5–6 μ, sublevibus, interdum disco suprahilari levi bene delimitato praeditis; pleurocystidiis nullis; cheilocystidiis ditopis; hyphis fibuligeris. Specimen typicum in Herb. Univ. Mich. conservatum: Smith n. *40858*.

Pileus 10–20 mm broad, obtusely campanulate, "hazel" to deep "tawny" on disc, paler over the broadly striate margin, surface moist but dull (not at all lubricous), "ochraceous tawny" faded; flesh fragile, soft, odor and taste not distinctive; lamellae distant to subdistant, broad, ventricose, ascending-adnate and soon seceding, about ochraceous tawny, edges fimbriate under a lens; stipe 10–20 mm long, ± 2 mm thick, equal, hollow, fragile, concolorous with gills above and with cap below, veil remnants faint, pallid.

Spores 10–13 × 5–6 μ, inequilateral in side view, ovate in face view, nearly smooth, plage faintly outlined in a few, tawny in KOH, with an apical callus; basidia 4-spored; pleurocystidia none; cheilocystidia abundant, of two types; fusoid-ventricose with elongated necks and obtuse apices, 42–60 × 8–10 μ; and elongated individuals with oval heads 8–12 μ broad and equal or merely slightly ventricose below; gill trama yellowish cinnamon in KOH, of enlarged more or less regularly arranged cells; pileus trama homogeneous, cinnamon in KOH, pigment-incrusted, clamp connections present.

Habit, habitat and distribution. Gregarious on tundra-like bank, below Gobler's Knob, Mt. Rainer National Park, Wash., Oct. 12, 1952. Additional collections were from Snow Lake.

Material examined. Sm-*40858*, TYPE; *40353*, 2-spored; *40351*, 2-spored; *40361*; *40865*.

Observations. The ferruginous to tawny pilei, distant gills, the stipe darker at the base than at apex, and long cheilocystidia with obtuse to capitate or oval apices are distinctive. In the 2-spored form from Snow Lake the spores measured 11–15 × 6–7.5 μ and were often obscurely angular in face view. In these the line marking the plage showed more readily than in the type. Occasional spores were forked, an abnormality not uncommon in *Coprinus* and *Psathyrella*.

19. **Galerina allospora** sp. nov.

Pileo 8–12 mm lato, acute conico demum campanulato vel plano-umbonato, glabro, hygrophano, cinnamomeo-brunneo demum fulvo, striato; lamellis confertis adnatis, angustis; stipite 20–30 mm longo, c. 1 mm crasso, fragili, sursum melleo, deorsum fusco-brunneo, sparse fibrilloso; sporis 9–12 × 5–6 μ; cheilocystidiis (35–)40–50(–69) × 9–13 μ, deorsum subventricosis. Specimen typicum in Herb. Univ. of Mich. conservatum; Smith n. *44038*.

Pileus 8–12 mm broad, sharply conic with a bent-in margin, becoming conic campanulate to expanded-umbonate, surface glabrous, moist,

hygrophanous, "cinnamon brown" on the umbo, "ochraceous tawny" and translucent-striate over the margin; flesh thin and fragile, odor and taste not recorded; lamellae close, ascending, adnate, narrow to moderately broad, ochraceous tawny, edges even or nearly so; stipe 20–30 mm long, ± 1 mm thick, equal, very fragile, with scattered pallid fibrils from a very thin veil, honey-color near apex, bister over basal part.

Spores 9–12 × 5–6 μ, slightly inequilateral in side view, in face view narrowly ovate, smooth but with a faint jagged line delineating the plage, many appearing obscurely angular near apex because of a slightly thickened band of wall material deposited in that zone, in optical section the spore appearing to have inconspicuous swellings—one opposite the other—, apical callus present; basidia 4-spored, not infrequently with yellow content as revived in KOH; pleurocystidia none seen; cheilocystidia abundant (35–)40–50(–60) × 9–13 μ, ventricose below and with a long neck and ± enlarged apex (7–9 μ in some), hyaline, thin-walled; hyphae incrusted with pigment; clamp connections present.

Habit, habitat and distribution. Gregarious on debris and dead sphagnum under spruce, Mud Lake Bog, Cheboygan County, Mich., Oct. 12, 1953. Known only from type locality.

Material examined. Smith-*44038*, TYPE; Sm-*44027; 44032; 44035; 44039*.

Observations. This species does not appear to be closely related to *G. naucorioides* and *G. tundrae*, the other dark brown species of this group. *G. naucorioides* has a convex pileus, is evenly dark-colored over pileus and stipe, and the spores do not show the curious sub-apical thickening. *G. tundrae* has more red in the fresh pileus and the stipe is not bister at the base in age but instead a dark red-brown. Its spores also lack the subapical thickened zone of wall material. The spore characters definitely recall those of *G. insignis* but the structure of the surface layer of the pileus, the absence of pleurocystidia, and the color of the fibrils on the stipe distinguish *G. allospora* from *G. insignis*.

20. **Galerina tsugae** sp. nov.

Pileo 5–12 mm lato, margine stricto, obtuse conico dein applanato vel late conico, cinnamomeo-brunneo, conspicue striato, primum strato tenui fibrilloso cinnamomeo-alutaceo vel pallide alutaceo obtecto ad marginem vel margine fibrillose fimbriato; lamellis distantibus, pileo concoloribus; stipite 10–20 mm longo, minus quam 1 mm diametro, pileo concolori primum fibrillis veli sparsis obtecto in parte inferiore; sporis 9–11 × 5–6 μ, levibus sed disco suprahilari levi delimitato instructis; pleurocystidiis nullis; cheilocystidiis 36–48 × 7–12 μ, apice longo flexuoso angustato praeditis, infra apicem ventricosis, pedicello hyalino vel fulvo praeditis; hyphis fibulatis; ad ligna putrida *Tsugae canadensis*. Specimen typicum in Herb. Univ. of Mich. conservatum; Smith n. *44070*.

Pileus 5–12 mm broad, obtusely conic with a straight margin, expanding to plane or remaining broadly conic, color "cinnamon-brown," hygrophanous, when faded near tawny on disc and paler over margin, conspicuously striate when moist, at first with a thin layer of fibrils over margin or margin faintly fibrillose-fringed, fibrils cinnamon-buff to pallid buff, soon glabrous; flesh very thin and fragile, no odor or taste; lamellae distant, narrow to moderately broad, adnate, concolorous with pileus; stipe 10–20 mm long, less than 1 mm thick, equal, concolorous with pileus over all or base slightly darker cinnamon-brown, lower portion with scattered fibrils from veil at first, glabrescent, apex pruinose.

Spores 9–11(–13) × 5–6 μ, narrowly inequilateral in side view, in face view narrowly ovate, rusty brown in KOH, smooth, but with a ragged line marking the suprahilar depression and occasional spores showing 1–2 small blisters (loosening of exosporium) about 3–4 μ back of apex; basidia 4-spored; pleurocystidia none but some basidioles retaining a dark brown pigment when revived in KOH; cheilocystidia 36–48 × 7–12 μ, ventricose above a hyaline to fulvous pedicel and with a long flexuous neck scarcely tapered to an obtuse apex; gill trama rusty brown from incrusting pigment; pileus trama homogeneous, darker in KOH than the gill trama, pigment incrusted; clamp connections present.

Habit, habitat and distribution. Gregarious along rotten hemlock logs, Mud Lake Bog, Cheybogan County, Mich.

Material examined. Sm-*44070*, TYPE; Sm-*44017*; *44028*.

Observations. This fungus has the stature of a small *G. triscopa* but lacks the acute umbo and has a well developed veil as well as darker colors. The microscopic characters, of course, are totally different. As to its relationships, it appears to be close to *G. vaccinii* in pigmentation and spore characters. This is, then, the only Eastern representative of the subsection, and it is interesting to note that it is the species with the least development of the fibrillose covering.

21. **Galerina mesites** sp. nov.

Pileo 10–20 mm lato, conico vel plano-umbonato, glabro, humido, hygrophano, in juvenilibus lubrico, ochraceo-brunneo unicolori, striatulo; odore haud manifeste acidulo, sapore miti; lamellis latis, breviter decurrentibus, ochraceo-brunneis; stipe 10–30 × 1–2 mm, unicolori, obscure castaneobrunneo, velo diffracto fibrilloso zona fibrillosae praedito, demum glabro nudoque; sporis 9–11 × 5–6.5 μ, subtiliter marmoratis discoque suprahilari levi delimitato praeditis; basidiis tetrasporis; pleurocystidiis hyalinis tenuitunicatis, ampullaceis apice subacuto praeditis; cheilocystidiis pleurocystidiis aequalibus; pellicula haud manifesta praesente, sed epicute ex hyphis hyalinis consistente; hyphis fibulatis; ad ligna frondosa putrida. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *44054*.

Pileus 10–20 mm broad, conic to plano-umbonate, surface glabrous, moist, hygrophanous (lubricous on young fresh caps), evenly tawny and translucent-striate part way to disc when moist, fading to cinnamon buff; flesh thin, pliant, odor faintly acidulous, taste mild; lamellae broad, short-decurrent, ± subdistant, tawny (both young and old), edges even to slightly fimbriate under a lens; stipe 10–30 mm long, 1–2 mm thick, equal, dark red brown over-all, with merely a faint fibrillose zone from broken veil, glabrous and naked in age.

Spores 9–11 × 5–6.5 μ, somewhat inequilateral in side view, narrowly ovate in face view, ochraceous tawny in KOH, deep red-brown in Melzer's reagent, wall faintly marbled (under oil) and with a smooth faintly delimited plage; basidia 28–34 × 6–7 μ, 4-spored; pleurocystidia scattered, 40–60 × 9–15 μ, fusoid-ventricose with subacute apices, hyaline, thin-walled; cheilocystidia similar to pleurocystidia, scattered; gill trama ± parallel but cells ± ellipsoid, epicutis subgelatinous (in KOH) but indistinct, consisting of a layer of hyaline hyphae 2–4 hyphae thick, pileus trama consisting of hyphae with yellow to rusty incrusting pigment; clamp connections present.

Habit, habitat and distribution. Closely gregarious on a hardwood log, Mackinaw City Hardwoods, Emmet County, Michigan. October.

Material examined. Sm-*44054*, TYPE.

Observations. This species is clearly close to *G. unicolor*. The cuticular subgelatinous hyphae are not appreciably narrower then the colored ones making up the trama beneath, and the pleurocystidia are similar in both. The spores are nearly smooth, however, and the veil is slight.

On this basis, it might be considered as belonging in the stirps *Cedretorum* where it would key out with *G. consobrina*, from which it differs in growing on hardwood, smoother spores which become dark red-brown in Melzer's solution, and the stipe becoming dark bay instead of bister.

22. **Galerina megalocystis** sp. nov.

Pileo 25–65 mm lato, viscido, brunneo vel ochraceo-brunneo; odore leniter farinaceo; sapore miti; lamellis adnatis dente exiguo decurrentibus, plus minusve 5 mm latis, pileo initio concoloribus; stipite 50–90 × 5–11 mm, fusco annulo fugaci praedito; sporis 8–10 × 5–6 μ, verrucoso-rugulosis, disco suprahilari levi bene evoluto praeditis; pleurocystidiis abundantibus, 60–90 × 10–15(–20) μ, ampullaceis, subacutis vel obtusis; cheilocystidiis pleurocystidiis simillimis, cellulis clavatis mucronatisve ad aciem nullis; epicute pelliculosa, ex hyphis hyalinis vel subhyalinis gelatinosis consistente; fibulis praesentibus; ad lignum putridum *Abietis*. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *3680*.

Pileus 25–65 mm broad, convex then plane or with a slight obtuse umbo, glabrous, viscid, margin translucent-striate when moist, "buckthorn-brown" to "ochraceous tawny" moist, hygrophanous, fading to "warm buff" or paler yellow, disc in some remaining darker; flesh thick in the disc, tapered to cap margin, watery brown fading to pale buff, odor slightly farinaceous when flesh is crushed, taste mild; lamellae bluntly adnate or with a slight decurrent tooth, soon seceding, equal, moderately close and broad (± 5 mm), concolorous with pileus young, darker and more rusty brown at maturity; stipe 50–90 mm long, 5–11 mm thick at apex, with a thin apical evanescent almost fibrillose annulus, equal to subclavate downward, hollow, surface grayish brown from dense longitudinally appressed fibrils giving it a somewhat striate appearance, base white mycelioid.

Spores 8–10 × 5–6 μ, oval in face view, inequilateral in side view, dark rusty brown in KOH, surface warty-rugulose except over the suprahilar depression, with a slight tendency for blisters to form near hilar end on standing in KOH; basidia 4-spored; pleurocystidia abundant, 60–90 × 10–15(–20) μ, fusoid-ventricose with long often wavy neck and obtuse to subacute apices, hyaline in KOH, thin-walled; cheilocystidia similar to pleurocystidia (fully as large), no clavate to mucronate cells present on gill edge; gill trama parallel or nearly so, yellow in KOH, hymenopodium and subhymenium not distinctive; cuticle a well-defined gelatinous pellicle of hyphae 3–5 μ in diam., hyaline or nearly so; trama of pileus ochraceous in KOH; clamp connections present.

Habit, habitat and distribution. Gregarious on log of *Abies*, Trinidad, Calif., Nov. 30, 1935, Parks and Smith.

Material examined. Smith-*3680*, TYPE; Sm-*42451*.

Observations. This is a robust species with exceptionally long, broad cystidia, mild taste and viscid pileus. The tips of the cystidia showed no tendency to enlarge—as is true of the type of *G. autumnalis*—and the pleurocystidia are more numerous as well as being much larger.

23. **Galerina subglabripes** sp. nov.

Pileo 10–15 mm lato, glabro, lamellis confertis, crenulatis; stipite 15 × 1 mm, pallide melleo, mox ad basin obscure rufescente brunneo vel biso, tenuiter pallido vel griseolo fibrilloso; sporis 7–9 × 4–4.5 μ, minutissime areolato-asperulis, disco suprahilari levi delimitato praesente; pleurocystidiis et cheilocystidiis praesentibus, illis subacutis; fibulis praesentibus; ad quisquilias. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *40459*.

Pileus 10–15 mm broad, convex-expanding to nearly plane, some with a slight umbo, surface glabrous, moist, hygrophanous, more or less dull tawny moist and slightly striate, fading to pinkish buff, when dried, darker; odor and taste not recorded; lamellae close, moderately broad,

adnate and with a slight tooth, dull cinnamon to dark ochraceous tawny, edges crenulate; stipe ± 15 mm long, ± 1 mm thick, equal, pale honey color and pruinose above, soon dark reddish brown to bister below and lower portion covered thinly with pallid to grayish fibrils, ± matted-fibrillose with pallid fibrils around the base.

Spores 7–9 × 4–4.5 μ, ovate in face view, in side view very obscurely inequilateral, appearing smooth but under oil the surface minutely areolate-roughened, plage delimited by a faint line, ochraceous-tawny to buckthorn brown in KOH; basidia 4-spored; pleurocystidia scattered, fusoid-ventricose to fusoid, 40–58 × 9–12(–16) μ, apices subacute; cheilocystidia (24–)28–55 × 8–11 μ, narrowly ventricose to nearly fusoid, hyaline or some with ochraceous walls in basal part, thin-walled; gill trama subparallel, pallid in KOH, subhymenium thin and not distinctive; pileus trama homogeneous, pallid to ochraceous in KOH; clamp connections present.

Habit, habitat and distribution. Gregarious on debris, Carbon River, Mt. Rainer National Park, Oct. 2, 1952. Known only from the Park.

Material examined. Smith-*40459*, TYPE.

Observations. This species was mistaken for *G. sideroides* in the field. It is very close to *G. rudericola* from which the lack of a fibrillose zone on the stipe distinguishes it. The appressed fibrils over the lower part of the stipe in *G. subglabripes* have not been demonstrated to be veil remnants.

24. **Galerina mollis** sp. nov.

Pileo 10–15 mm lato, conico demum conico-umbonato, glabro, hygrophano, pallide fulvo; lamellis confertis, angustis, adnatis, stipite 30–35 mm longo, 1.5 mm crasso, fibrilloso-annulato, deorsum brunneo; sporis 7–9 × 4.5–5.5 μ; pleurocystidiis 34–56 × 12–18 μ; ad detritum *Capnoidis schuleri*. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *41074*.

Pileus 10–15 mm broad, conic becoming conic-umbonate, the margin often spreading, glabrous, moist, hygrophanous, ochraceous tawny and striate to umbo, buff when faded; flesh very soft, odor and taste mild; lamellae close, narrow, adnate, edges even, about concolorous with pileus; stipe 30–35 mm long ± 1.5 mm thick, equal, uneven, fragile, concolorous with gills, at base pallid-silky, with a very inconspicuous superior fibrillose annulus, base darkening to watery brown where bruised.

Spores 7–9 × 4.5–5.5 μ, inequilateral in side view, in face view ovate to oval, tawny in KOH, outer wall loosening around the depression, which is smooth, remainder of surface minutely warted; basidia 4-spored; pleurocystidia scattered, 34–56 × 12–18 μ, ventricose with short necks and obtuse to rounded apices; cheilocystidia 40–58 × 10–15 μ, fusoid-ventricose with obtuse apices; gill trama subparallel, hyaline or nearly

so in KOH; pileus trama homogeneous, hyaline to pale ochraceous in KOH; clamp connections present.

Habit, habitat and distribution. Scattered on remains of herbaceous stems (of *Capnoides*), Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952.

Material examined. Smith-*41074*, TYPE; Sm-*41077*; *41082*; *41083*; *41100*.

Observations. The very soft flesh, broadly ventricose pleurocystidia and the change in color of the base of the stipe when bruised are distinctive. The veil remnants, though forming a thin zone at first, soon vanish. In many respects this species connects the stirps *vittaeformis* with stirps *cedretorum*. The habitat is most unusual for a *Galerina*. The carpophores occur along the old stems singly in the manner of many species of *Psilocybe* which have a similar type of habitat.

25. **Galerina latispora** sp. nov.

Pileo 10–15 mm lato, convexo, glabro, hygrophano, fulvo; lamellis subdistantibus, adnatis; stipite 15–25 mm longo, 1–2 mm crasso, glabro; sporis 9–11 × 6.5–7.5 μ (12–13 × 8–8.5 μ); pleurocystidiis 42–60 × 7–12 μ, subventricosis. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *40099*.

Pileus 10–15 mm broad, convex, expanding to broadly convex, surface glabrous, moist, hygrophanous, striatulate moist, "tawny" and fading to dingy tan; lamellae near tawny at maturity, subdistant, narrow to moderately broad, nearly horizontal and bluntly adnate; stipe 15–25 mm long, 1–2 mm thick, sometimes narrowed downward, apex faintly pruinose, lower portion naked, tawny over all.

Spores 9–11 × 6.5–7.5 μ (12–13 × 8–8.5 μ), broadly ovate or in many the apex snoutlike, broadly inequilateral in side view, tawny or darker in KOH, very minutely rugulose-roughened and with a smooth suprahilar depression marked by only a faint ragged line of granules; basidia 2- and 4-spored; pleurocystidia scattered to rare, similar to the cheilocystidia; cheilocystidia abundant, 42–60 × 7–12 μ, subfusoid with flexuous walls in the neck and subacute to subcapitate apices, hyaline, thin-walled; gill trama tawny in KOH, somewhat interwoven; pileus trama tawny in KOH, homogeneous; pigment incrusted on hyphae of carpophore; clamp connections present.

Habit, habitat and distribution. Scattered on moss in wet areas along a stream, Castle Peak, Mt. Rainier National Park, Wash., Sept. 20, 1952. Known only from the type locality.

Material examined. Smith-*40099*, TYPE.

Observations. The very broad, almost smooth spores, rare to scat-

tered pleurocystidia, evenly colored carpophores and convex pileus are distinctive. This species differs from *G. oreina* mainly in its nearly smooth spores with an indistinctly marked smooth plage. The spores are larger and with somewhat thicker walls in addition. The absence of a veil may be an additional character, but more material in younger stages of development is needed to verify this. In the revived material studied to date, the cheilocystidia have been found to be hyaline, but in the hymenium many of the old basidia have tawny walls in the lower portion, and some tawny brown basidioles are also present.

26. **Galerina inconspicua** sp. nov.

Pileo 6 mm lato, convexo, umbonato, glabro, alutaceo vel ochraceo-brunneo; lamellis cum pileo concoloribus, ventricosis, latis, distantibus; stipe 10 × 2 mm, cum pileo concolori sed ad basin fusco, apice pruinato, subtus e velo fibrilloso sed haud annulato; sporis 11–12 × 6–6.3 μ, admodum subtiliter punctatis vel marmoratis vel sublevibus, disco suprahilari bene delimitato instructis; basidiis 2-sporis; pleurocystidiis ampullaceis, 48–62 × 7–15 μ; cheilocystidiis aut pleurocystidiis simillimis aut vesiculosis vel clavatis; hyphis fibuligeris. Inter *Polytricha* in silva sparsa *Nothofaginea* Patagoniae, Argentina; Singer n. M-662.

Pileus 6 mm broad and 5 mm high, convex and umbonate, glabrous, hygrophanous, buff to light ochraceous brown (between *buff* and *gold leaf*), striatulate when wet, fading when dry; flesh thin, without distinct odor, taste not recorded; lamellae concolorous with pileus, ventricose and broad, adnexed to adnate, distant; stipe 10 mm long, 2 mm thick, equal or very slightly tapering downward, concolorous with the pileus but the base brown, pallid pruinate at the apex, below the apex not pruinate but pallid fibrillose from the distinct but never annulate veil, no veil remnants on margin of pileus.

Spores 11–12 × 6–6.3 μ, fewer up to 15 μ long, some up to 7.5 μ broad, very finely punctulate or marbled, almost smooth in a minority, with well marked plage, moderately deep rusty-brown in KOH, subfusoid in front view, slightly inequilateral in side view, with a strong callus; basidia 2-spored, with few 1-spored intermixed, 24–27 × 8–8.5 μ, more rarely up to 9.7 μ broad; pleurocystidia hyaline to pale brownish in KOH and NH_4OH, ventricose below, ampullaceous with medium thin and long-cylindrical neck, usually not thickened at the apex, thin-walled, 48–62 × 7–15 μ; cheilocystidia like the pleurocystidia or merely vesiculose to clavate without a neck; caulocystidia in dense bunches, hyaline to melleous, ventricose at the base, filamentous in the upper portion, much like the pleurocystidia, 42–83 × 8–12.5 μ, gill trama regular, melleous to light brownish but without distinct pigment-incrustations; all hyphae with clamp connections.

Habit, habitat and distribution. Scattered among *Polytrichum* in an opening of the *Nothofagus dombeyi* woods, May 16, 1952, Quetrihue, Nahuel Huapi National Park, Terr. Neuquen, Patagonia, Argentina.

Material examined. Singer, M-*662*, TYPE (LIL).

Observations. This species differs from *G. subannulata*, which is otherwise similar, in having the veil somewhat less abundantly developed, in somewhat less developed exosporial ornamentation, in non-mucronate spores which are somewhat paler in ammonia and definitely narrower, also in having usually ampullaceous pleurocystidia with well developed and long cylindrical necks and finally in the lighter ochraceous buff color of the pileus which is umbonate rather than obtuse. The spores are too large to allow it to be considered a bispored *G. oreina*, which, in addition, has a darker pileus and different cheilocystidia, also a much more elongate stipe.

27. **Galerina oreina** sp. nov.

Pileo 3–8 mm lato, ochraceo-brunneo vel obscure ochraceo-brunneo; lamellis distantibus, latis, obtuse adnatis; stipite 20–40 × 0.5–0.75 mm, pallidiore pileo, ad basin haud sensim obscuriore; velo rudimentario dein fugaci; sporis 7–9 × 5.5–6.3 μ, asperulatis, disco suprahilari levi delimitato praeditis, basidiis tetrasporis; pleurocystidiis sparsis, fusiforme ventricosis, ampullaceis, apice longo subacuto praeditis; cheilocystidiis pleurocystidiis simillimis sed multis ex eis ad basin ochraceis membranis subincrassatis praeditis; hyphis fibulatis; ad muscos prope glaciem. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *40344*.

Pileus 3–8 mm broad, obtuse to convex with a straight margin, remaining unexpanded, tawny to dark ochraceous tawny and with broad translucent striations, fading to buffy tan or paler; flesh soft and fragile, odor and taste not distinctive; lamellae distant, broad, bluntly adnate but soon seceding, concolorous with pileus; stipe 20–40 mm long, 0.5–0.75 mm thick, equal, flexuous, paler ochraceous tawny than pileus, base not appreciably darker (often paler where buried in moss), veil rudimentary and all traces soon gone.

Spores 7–9 × 5.5–6.3 μ, in face view broadly ovate, in side view inequilateral, dark rusty in KOH, wall roughened and depression marked by a ragged line; basidia 4-spored; pleurocystidia scattered, fusoid-ventricose, 40–60 × 10–15 μ, with long narrow (4–6 μ) necks and subacute apices; cheilocystidia similar to pleurocystidia, but many with the basal part having ochraceous slightly thickened walls in KOH; gill trama of enlarged hyphal cells, subregular, pale tawny in KOH; pileus trama homogeneous, tawny to rusty in KOH, pigment incrusted; clamp connections present; none or very few caulocystidia present on the stipes examined.

Habit, habitat and distribution. Gregarious on moss in areas made moist by runoff from nearby glaciers and snowfields, Snow Lake, Mt. Rainier National Park, Wash.

Material examined. Smith-*40344*, TYPE; Sm-*39809; 39810; 40102; 40134; 40258; 40356; 40358; 40359; 40369; 40370; 40630; 40819.*

Observations. The distinctive characters are the distant gills, typically non-darkening stipe, pleurocystidia, broad short spores and tawny pileus. The colored, slightly thickened walls of the lower portion of the cheilocystidia are rather noteworthy characters in revived material. In Sm-*40258* the cheilocystidia average longer than in the other collections and have more of a colloidal content. The broad spores place the collection here. In Sm-*40102* the shape of the cheilocystidia is highly variable. No veil was noted in this collection, and a few caulocystidia were found. *G. oreina* is most closely related to *G. minima* but the broad dark spores distinguish it along with the more abundant larger pleurocystidia.

In some collections, Sm-*40300* for example, the stipe darkened somewhat, becoming bay brown below, and the pleurocystidia were narrower (8–12 μ). It may be possible that a distinct variety exists based on these characters but more data are needed.

28. **Galerina funariae** sp. nov.

Pileo 10–30 mm lato, ochraceo-brunneo; lamellis subdistantibus, latissimis, adnatis, ochraceo-brunneis; stipite 20–35 × 1–1.5 mm cum pileo concolori vel pallidiore, demum biso in parte basali, apice pruinato, ceterum glabro; sporis 8–10 × 5–6 μ, minute asperulatis vel rugulosis, depressione suprahilari levi praesentibus; basidiis tetrasporis; pleurocystidiis sparsis, fusoideo-ventricosis, apice angustatis obtusis vel subacutis; cheilocystidiis pleurocystidiis simillimis vel elongatioribus; dermatocystidiis stipitis fusoideis; fibulis praesentibus; locis exustis prope *Funariam*. Specimen typicum in Herb. Univ. Mich. conservatum; Smith n. *12063*.

Pileus 10–30 mm broad, obtuse at first, becoming convex to nearly plane, surface glabrous, moist, hygrophanous, with broad translucent striations extending to the disc, "tawny" to "ochraceous tawny" and fading to sordid ochraceous buff, margin usually paler than disc, smooth; flesh concolorous with surface, watery and fragile, odor and taste not distinctive; lamellae subdistant, (10–15 reach the stipe), very broad (8–10 mm), adnate, oval in outline, 1–2 tiers of lamellulae, pale tawny when young, concolorous with cap in age; stipe 20–35 mm long, 1–1.5 mm thick, pruinose above from caulocystidia, fragile, equal, concolorous with cap or paler, becoming bister from the base upward, very scantily mycelioid at base, lower half glabrous.

Spores 8–10 × 5–6 μ, subinequilateral in side view, in face view broadly ovate, ochraceous tawny to slightly darker in KOH, exosporium

minutely roughened to rugulose, with a smooth plage, apical callus present; basidia 4-spored, 24–26 × 8–9 μ, hyaline in KOH; pleurocystidia scattered, 35–50(–56) × 8–12 μ, fusoid-ventricose, thin-walled, hyaline in KOH, apex obtuse to subacute; cheilocystidia similar to pleurocystidia or slightly more elongated; gill trama subparallel, pale ochraceous from incrusting pigment; pileus trama homogeneous, darker than gill trama; caulocystidia fusoid; clamp connections present.

Habit, habitat and distribution. Scattered near *Funaria* and on it in a burned area (muck soil as base), Kalaloch, Wash., April. One collection was from a fresh grass seeding over the burn in which some moss was also present.

Material examined. Smith-*12063*, TYPE; Sm-*13001*.

Observations. This is a large *Galerina* distinguished by the stipe, which becomes distinctly darker below in age, the lack of a veil, the habitat and pale minutely roughened spores. It is distinguished from *G. minima* by lack of a veil, paler pileus, distinctly darkening stipe, and much larger size.

29. **Galerina thujina** sp. nov.

Pileo pallide cinnamomeo-brunneo, 8–10 mm lato; lamellis distantibus vel subdistanibus; stipite obscure cinnamomeo-fusco ad basin, tenuiter pallide velato, apice pruinoso, c. 10 × 1 mm; sporis 6.5–8 × 4–5.5 μ, subcompressis, minutissime asperulatis, *disco* levi delimitato; pleurocystidiis sparsis 50–70 × 9–13 μ fusoideo-ventricosis apiceque acutis vel ramosis biramosisve, intus granulosis; cheilocystidiis pleurocystidiorum modo ramosis; hyphis fibulatis. Ad truncum *Thujae*. Specimen typicum in Herb. Univ. of Michigan conservatum; Smith n. *42589*.

Pileus 8–10 mm broad, plano-umbonate, margin bent in somewhat at first, surface glabrous, moist, hygrophanous, pale "cinnamon-brown" and slightly translucent-striate moist, dingy tan when faded; flesh thin, fragile, odor none, taste not recorded; lamellae distant to subdistant, broad, depressed-adnate, pale ochraceous becoming more or less tawny or nearly concolorous with the pileus; stipe ± 10 mm long, ± 1 mm thick, equal, faintly fibrillose over lower part from remains of a thin pallid veil, very dark cinnamon-brown at base, paler upward, apex pruinose.

Spores 6.5–8 × 4–4.5 × 5–5.5 μ, compressed slightly, subelliptic in side view, ovate in face view, very minutely wrinkled and with a ragged line marking the plage, occasionally the outer layer of the spore wall separating to form small blisters—usually around the plage area—, color in KOH pale cinnamon-brown to dingy tawny; basidia 4-spored, 20–23 × 6–7.5 μ; pleurocystidia scattered, 50–70 × 9–13 μ, fusoid-ventricose with subacute apices or the neck branched and at times primary branches giving rise to secondaries—branching mostly dichotomous at

times—the apices more or less spearhead-like in shape, content of cystidia granular in water mounts of fresh material, hyaline and homogeneous in KOH, walls thin and hyaline; cheilocystidia similar to pleurocystidia, with more of a tendency toward branching (?); hyphae of pileus trama heavily incrusted with pigment, clamp connections present.

Habit, habitat and distribution. Scattered on an old cedar (*Thuja occidentalis*) log, Tahquamenon Falls State Park, Mich., Sept. 11, 1953.

Material examined. Smith *42589*, TYPE.

Observations. The small spores have an outer layer which is about as separable as that of *G. decipiens* f. *separans* or less so. The unusual characters of this species are the forked cystidia and slightly compressed spores. The darkening stipe and cinnamon-brown pileus along with the habitat on *Thuja* appear to be the distinguishing field characters.

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LEO ROY TEHON
1895–1954

LELAND SHANOR

(WITH PORTRAIT)

His sudden and unexpected death on October 17, 1954, at the age of 59, brought to a close prematurely the long and useful career of Dr. Leo Roy Tehon, since 1921 Botanist of the Illinois Natural History Survey and since 1935 Head, Section of Applied Botany and Plant Pathology. Dr. Tehon was a charter member of the Mycological Society of America. In addition to mycology, he was interested in the taxonomy and distribution of vascular plants, diseases of vascular plants, especially the diseases of forest and shade trees, and other areas of botany. These varied interests are indicated by his numerous publications on a number of botanical subjects. Many of these publications are in the form of circulars and bulletins intended to place botanical information before the people of his state and the general public.

Leo Roy Tehon was born in the small town of Dumont, South Dakota, on June 21, 1895. He was the son of Patrick John Tehon, a native of Ireland who came to the United States as a youth, and the former Bertha May Whittier, a native of Nebraska and a distant relative of the distinguished American poet, John Greenleaf Whittier. Throughout Leo's boyhood his family had very limited income. His father died when Leo was quite young so that very early he had to seek employment to help meet family expenses. He worked as a brakeman on the railroad to help finance his early schooling. On April 13, 1918, he married Mary Viola Bruner of Mattoon, Illinois, and from this marriage there were born two children, Stephen Whittier Tehon, on October 20, 1920, and Atha Lee Tehon, on January 20, 1926. Mrs. Leo Tehon died on May 5, 1953. His son and daughter survive him, as do his mother and two sisters.

The early education that Dr. Tehon received was interrupted from time to time. He attended the Catholic school at Sturgis, South Dakota, and public schools of Deadwood, South Dakota, and of Fremont, Nebraska. He matriculated in high school first at Fremont, Nebraska, and later at Sheridan, Wyoming. His undergraduate college training began

LEO ROY TEHON

at Fremont Normal School at Fremont, Nebraska, was continued at Gregg School in Chicago, and completed at the University of Wyoming, from which he received his Bachelor of Arts degree in 1916. It was at the University of Wyoming that Leo became interested in botany,

due largely to the stimulating influence of Professor Aven Nelson. It was here that he made his decision to pursue a career in the field of botany. He held an assistantship in botany at Wyoming during his senior year at the University.

After completing his work at Wyoming, Leo became, for the year 1916–1917, an assistant in the Department of Botany at the University of Illinois and commenced his graduate study. After one year he found it necessary to delay further study at that time and accepted a position for the year 1917–1918 as a teacher of botany in the Arsenal Technical High School, Indianapolis, Indiana. He served in the Army 1918–1919, first in the infantry and later, after attending the Army Laboratory School at Yale University, in the Medical Corps. He received an honorable discharge in 1919 and returned to Illinois as Assistant Plant Pathologist, United States Department of Agriculture, in charge of barberry eradication in the state, serving in this capacity during 1919–1920 and 1921–1922. During the year between these two assignments, 1920–1921, he was employed by the Mount Arbor Nurseries of Shenandoah, Iowa.

Dr. Tehon received his appointment to the staff of the Illinois Natural History Survey on July 1, 1921, and remained on the staff of the Survey until his death, except for one year, 1924–1925, when he took leave to devote more time to work toward an advanced degree. Since 1921 he had served as Collaborator, Plant Disease Survey, United States Department of Agriculture. He obtained the degree of Master of Arts from the University of Illinois in 1920 and continued graduate study on his doctorate under the direction of Professor Frank Lincoln Stevens while holding his position with the Survey. He received his degree of Doctor of Philosophy from the University of Illinois in 1934. He was appointed Head, Section of Applied Botany and Plant Pathology of the Survey in 1935. From 1924 to 1942 he was a consultant of the Davey Tree Expert Company, and from 1945 to 1947 was Acting Chief of the Illinois Natural History Survey. In 1947 he was appointed Research Professor of Plant Pathology in the Graduate College of the University of Illinois to offer a course on shade and forest tree diseases and to direct research of graduate students interested in this area of study. Since 1948 he had been a member of the administrative committee appointed by the Graduate College to supervise the curriculum for advanced degrees in plant pathology at the University.

Leo Tehon was a rather shy, modest, sensitive man and deeply interested in the welfare of his community and of his associates. He loved classical music and played the violin for his own enjoyment and relaxa-

tion. He was a student of pioneer history and interested in foreign languages. One of his last contributions was a translation from the Italian of Tozzetti's paper of 1767 on the true nature, causes and sad effects of the rust, the bunt, the smut, and other maladies of wheat and of oats in the field, published as Phytopathological Classics No. 9, American Phytopathological Society. Dr. Tehon was especially active in the work of the Boy Scouts in his community and served the organization in a number of capacities, for two years as President of the Arrowhead Council. His further interest in the development of young people was evidenced by his contributions to the work of the Junior Academy of the Illinois State Academy of Science and his active participation as a member of the Science Talent Search Committee of the State Academy. His other community interests were principally in the Community Chest and the work of various committees and commissions, to which he was usually appointed, established to fight the invasion of or to attempt to control such epidemic tree diseases as phloem necrosis of elms, Dutch elm disease, and oak wilt. He was always willing and anxious to give generously of his time to serve his community in any way his talents and experience would be of value. He was a member of the Champaign Exchange Club, serving as president in 1932, and belonged to the American Legion. He was a member of the First Presbyterian Church of Urbana and active in its affairs.

Dr. Tehon was a member of a number of scientific and professional organizations, in addition to the Mycological Society of America. He was a Fellow of the American Association for the Advancement of Science, a member of the Botanical Society of America, of the American Forestry Association, of the Torrey Botanical Club, of the Ecological Society of America, of Nature Conservancy, of the American Phytopathological Society, of Phi Beta Kappa, of the Illinois State Academy of Science (which he served as Secretary from 1943 to 1946 and as President 1946–1947), of the Shade Tree Conference (which he served as a member of the Executive Committee from 1944 to the time of his death), and of the Midwest Chapter of the Shade Tree Conference (which he served as Vice-President in 1953 and of which he was President at the time of his passing). He also served from time to time on important committees of many of these organizations. For the years 1945 and 1946 he was a member of the editorial board of "Phytopathology" and for several terms served on the Editorial Committee of the "Transactions of the Illinois State Academy of Science."

Dr. Tehon's research interests were quite varied, including taxonomy and distribution of fungi, diseases of plants of economic importance, anti-

biotics, plant disease surveys, aromatic fluorinated compounds as fungicides, poisonous plants, taxonomy and distribution of vascular plants, and others. He wrote extensively on these subjects and his list of publications numbers almost 90 titles. The following selected bibliography includes only those more important titles pertaining to work of a clearly mycological nature. Those dealing with other areas, for example plant pathological writings, have not been included, nor have those that might be regarded as primarily popularization of botanical subjects in which he was interested.

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NOTES AND BRIEF ARTICLES

A new genus of Dematiaceae [1]

In 1822, Persoon (Myc. Eur. **1**: 38. 1822) erected the genus *Spicularia* with descriptions of six species. The genus was reduced to synonymy by Fries (Syst. Myc. **3**: 339. 1829) with removal of the six species to *Botrytis*.

In 1870, Fuckel (Jahrb. Nas. Ver. Naturk. **23–24**: 359. 1870) used the name *Spicularia* for a fungus which he designated *S. Icterus*. Fuckel's species, as described and illustrated, does not seem to be a *Spicularia* in the sense of Persoon, since the conidiophores are shown as distinctly dark and the spores, while described as continuous (?), were probably, as vaguely suggested in the illustration, 2-celled.

The name was next used by Timonin (Can. Jour. Res. **18C**: 314. 1940) who emended Fuckel's description. Timonin isolated a fungus from the rhizosphere of alfalfa which closely resembled the description and illustration of *Spicularia Icterus* Fuckel. A search for Fuckel's specimen was instituted by Timonin but no fungus meeting this description was found on any of Fuckel's collections in any herbarium in North America or at the Imperial Mycological Institute, Kew, England.

Lindau (Rab. Krypt. Fl. **8**: 120. 1907) doubted the accuracy of Fuckel's illustration and even Fuckel was not positive about the septation of the spores, as may be inferred from his characterization of the species by "conidiis . . . simplicibus (?)." On this evidence, Timonin decided that his fungus and Fuckel's fungus were at least congeneric and thereupon emended the description of Fuckel's genus to include the new species, *S. terrestris*.

It is evident that Timonin emended the misapplication of Fuckel, and since *Spicularia* cannot be used for a fungus possessing the characters of *S. terrestris*, a new genus is proposed to accommodate this and other species having similar characters.

Umbellula gen. nov.

Stipites conidiophorum simplices vel maturitate ramosi, erecti, dilute fuliginei, septati, summo apicis ramos obclavatos radiatim fasciculatos modo umbellae ferentes; conidia in apicibus inflatis ramorum nascentia in spiculis, dilute fuliginea, uniseptata.

Typus: *Spicularia terrestris* Timonin, Can. Jour. Res. **18C**: 314. 1940.

[1] Excerpt from thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the State University of Iowa.

Stalks of conidiophores simple, or sometimes branched, especially in old cultures, erect, septate, dark, bearing at tip an umbelliform cluster of branchlets, each branchlet ending in a vesicular swelling on which conidia are borne singly on spicules, later proliferating at tip; conidia fuliginous, 1-septate.

Umbellula terrestris (Timonin) comb. nov.

Spicularia terrestris Timonin, Can. Jour. Res. **18C**: 314. 1940.

Stalks of conidiophores simple or occasionally branched, erect, septate, dilute fuliginous above, darker below, arising from a swollen basal cell to which are attached 1–4 swollen root-like cells which merge into the slender hyphae of the mycelium, up to 500 μ tall, 4–5 μ in diameter above basal cell, terminating in a more or less swollen tip from which arise 6–12, occasionally fewer or more, obclavate branchlets arranged in an umbel, these 14–18 μ long, 2.5–3 μ in diameter at base, each tipped by a globose swelling (up to 8 μ) bearing 15–20 spicules on which conidia are borne singly, proliferating irregularly in old cultures; conidia dry, cylindrical-oval, smooth, pale fuliginous, 1-septate, slightly constricted at septum, 7–8 $\times$ 2.5–3 μ.

Dark brown chlamydospores terminal or intercalary in chains on old submerged hyphae, globose (3–6 μ in diameter) to oval or nearly cylindrical, 7 $\times$ 4 μ.

Specimens examined: Panama Canal Zone: Barro Colorado Island, on palm sheath collected 18 July 1952, put in moist chamber 12 March 1953, GWM *8739;* Fort Sherman area, on leaf of coconut palm, collected 26 August 1952, put in moist chamber September, 1952, GWM *8736*. In addition to the two collections cited, which were secured in pure culture, Dr. Wendell M. Farrow (Mycologia **46**: 632–646. 1954) isolated the species (Unknown #*4*) twice from Barro Colorado Island soils and it has appeared repeatedly on litter of all sorts from Barro Colorado Island, especially angiospermous wood and bark when placed in moist chambers. Dr. S. C. Damon isolated and identified the species from an apple twig collected by Dr. G. W. Martin in Iowa City, 1952. Mr. Robert Bandoni (*54–20*) isolated the fungus from angiospermous wood collected at Iowa City in 1954. What appears to be the same species, isolated from soils in the Belgian Congo, was recently received from Dr. A. van Beverwijk, Baarn, Netherlands.

A culture of Timonin's fungus was obtained from the American Type Culture Collection. The conidiophore stalks of the type culture branch profusely. This fact is not mentioned in Timonin's paper and also is not

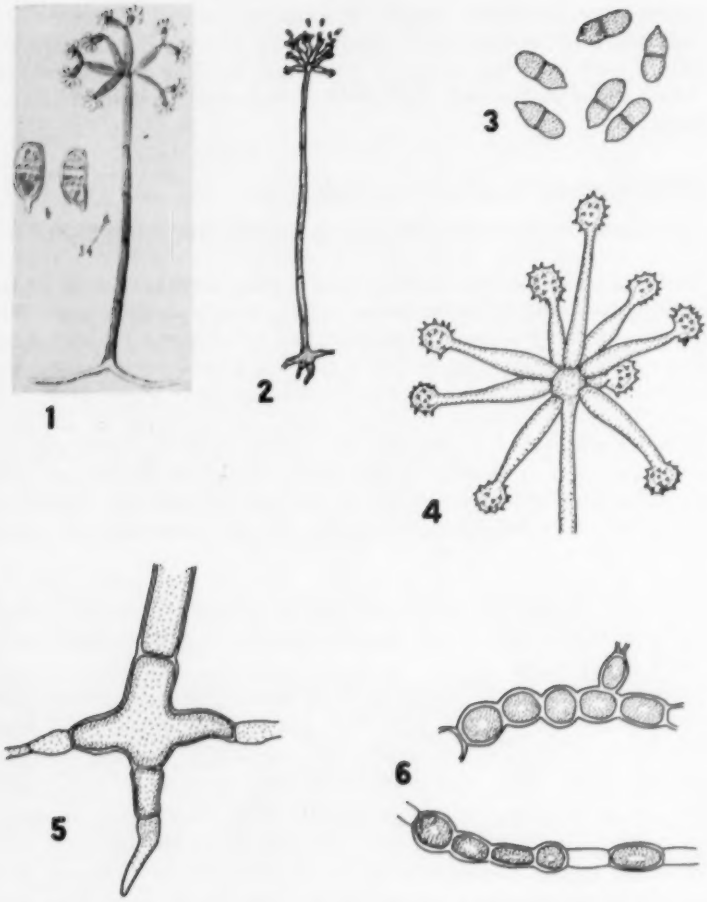


FIG. 1. *Spicularia Icterus.* Photograph of Fuckel's figure. FIGS. 2-6. *Umbellula terrestris.* 2. Habit, × 120; 3. Conidia, × 1000; 4. Umbel of conidiophore branches (drawn by G. W. Martin), × 1000; 5. Basal cell of conidiophore with root-like appendage, × 1000; 6. Chlamydospores, × 1000.

characteristic of the other specimens examined. Since this has been in continuous culture since 1939, it is possible that its characteristics may have altered. Its cultural characteristics were compared with those of the Barro Colorado, Belgian Congo, Bandoni's Iowa City and Martin's Iowa City specimens. All of the cultures formed a pale yellow, slowly-

spreading growth in the medium in seven days. The Barro Colorado isolate turned the medium black after the first week and formed conidiophores abundantly over the entire surface of the medium. The specimen collected by Bandoni at Iowa City turned the medium gray and also formed conidiophores abundantly. The other three cultures remained pale yellow and formed conidiophores very sparsely, mostly near the margins of the growths. The latter three formed very few chlamydospores. The Barro Colorado and Bandoni cultures formed numerous chlamydospores which may have been the cause of their dark appearance. The same results occurred when the specimens were re-cultured. Although they differed in culture, there seemed to be no distinct morphological differences in the specimens.

If Fuckel's specimen is ever found and proves to be the same as *Umbellula*, then *Spicularia Icterus* will have to be transferred to *Umbellula*. In that case, it will become the second species in the genus or, if identical, its specific epithet will have to replace that of Timonin.

Fuckel's original description is as follows:

Spicularia Icterus Fuckel, Jahrb. Nas. Ver. Naturk. **23–24**: 359. 1870. Pl. II, Fig. 34.

Caespitibus laxis, in macula exarida; hyphis fructiferis erectis, lineam altis, septatis, fuscis, apice subdichotomo-ramosis, ramulis apice conidia capitata gerentibus; conidiis candidis, oblongo-ovatis, breviter stipitatis, 14 Mik. long., 8 Mik. crass., simplicibus (?).

The similarities of the illustration of Fuckel reproduced here (FIG. 1) and the illustrations of *Umbellula terrestris* are rather obvious. The main difference is in the character of the spores. Fuckel gave $14 \times 8\,\mu$ as the size of the spores and referred to them as clear, unseptate (?). The spores of *U. terrestris* are smaller ($7–8 \times 2.5–3\,\mu$), dark and 1-septate.

In Saccardo's system, *Umbellula* is in the Macronemeae, Didymosporae group of the Dematiaceae.

I wish to extend my appreciation to Dr. Donald P. Rogers for checking and correcting the Latin diagnosis.

This work was done in the Mycological Laboratory of the State University of Iowa under the supervision of Professor G. W. Martin.—EVERETT F. MORRIS, Botany Department, State University of Iowa, Iowa City, Iowa.

ON SPHAERODERMA EPISPHAERIA

Surface litter under beeches, collected at Box Hill, Surrey, England, in August, 1954, and placed in a moist chamber in late September of the same year, yielded various fungi, among them a pyrenomycete with a translucent gelatinous wall, later appearing black because of the mass of large, dark, flattened, lemon-shaped spores which filled the interior. Asci were not observed in the original material but the spores appeared to be ascospores and this proved to be the case when the fungus was secured in pure culture (G.W.M. *8874*). It grows satisfactorily on weak oat agar, on grass stems in plain agar and on sterilized rabbit dung, and rather erratically on corn stalks moistened with a weak solution of mineral salts. As expected, asci are present, but gelatinize early, well before the maturity of the spores (FIG. 1, A), and the latter attain their full size, characteristic shape and netted surface while still immersed in the gelatinous interior of the ascocarp, eventually emerging in a long black tendril, in both respects suggesting *Chaetomium*. A papillate ostiole is present from early stages. At maturity the ascocarps are yellow, with thick translucent, gelatinous walls, the black mass of spores showing through the walls, mostly from 250 to 400 μ in diameter, with a definite fimbriate ostiole 90–110 μ long (FIG. 1, B, C). The mature spores are nearly black, somewhat flattened, lemon-shaped in side view, but broadly fusoid when seen from the edge, and mostly fall within the dimensions 16–20 × 11–13 × 9–10 μ, but some smaller and some larger spores are present in most mounts. The reticulation is rather regularly netted on the wide face, but the meshes tend to be long and narrow when seen from the edges.

Except for the spore size, the fungus agrees reasonably well with the description of *Sphaeroderma episphaeria* (Phill. & Plowr.) Sacc., as given by Petch (Trans. British Mycol. Soc. **21**: 254. 1938). Petch gives the spore size as 25–34 × 12–18 μ. The original description of *Melanospora episphaeria* Phill. & Plowr. (Grevillea **10**: 71, pl. 158, f. 2, a-d. 1881) says ".03 mm. long by .01 to .012 wide," i.e. 30 × 10–12 μ. Petch cites five synonyms. One is, of course, the original name given by Phillips and Plowright; two others are changes by Clements, 1909, and by Clements and Shear, 1931, and need not be further considered. A fourth is *Sphaeroderma epimyces* Höhnel (Sitz. k. Akad. Wien, I. Abt. **116**: 103. 1907), which is said to have been found on the stroma of a *Hypomyces*, as was the original collection of Phillips and Plowright, as well as a French specimen they cite, but the spores were described as flattened, and the dimensions are given as 24–28 × 13–18 × 11–12 μ. The fifth synonym is *Microthecium episphaerium* (Phill. & Plowr.) Höhnel

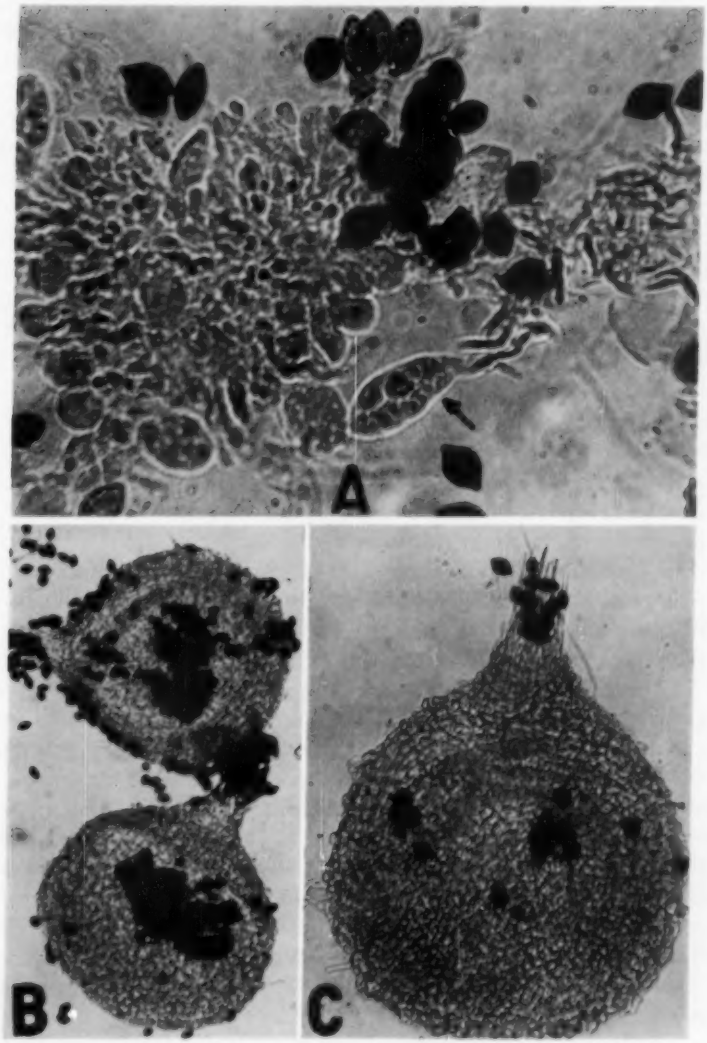


FIG. 1. *Sphaeroderma episphaeria*. A. Tuft of asci from base of ascocarp. The arrow indicates an ascus in early stage of dehiscence, × 400. B. Two mature ascocarps. These were bearing spore tendrils when mounted and many spores remain within, × 125. C. A nearly empty ascocarps, showing the beak and fimbriate ostiole, × 180.

(Sitz. k. Akad. Wiss. Wien, I. Abt. **123**: 98. 1914). Höhnel believed that *Microthecium* Corda, 1842, was the same as *Sphaeroderma* Fuckel, 1870. Corda's genus was described as non-ostiolate and Höhnel stresses this character. Tulasne, in the Fungi Hypogaei, 1851, which I have not seen, described what he thought was Corda's species, but Höhnel believed that Tulasne was mistaken. Grove (Jour. Bot. **74**: 191–193. 1936) discusses the genus and concludes that it is not at present possible to decide whether it is the same as *Microthecium*.

There was no trace of a *Hypomyces* in the litter from which the Box Hill isolate was made. When inoculated on sterilized sections of corn stalks, sometimes no growth is made, sometimes only mycelium appears without ascocarps after as long as 2 months, sometimes a membranous subiculum is formed, looking much like a *Hypomyces* stroma, against which the ascocarps stand out, appearing red, then black to the naked eye. The difference in growth appears to be correlated with the maturity of the stems, the best growth appearing on stems cut while still green. On weak oat agar and on grass agar this subiculum is completely lacking and the ascocarps develop on the surface of the agar, with practically no subaerial mycelium, as do many Sordarias.

Despite the smaller spores, and bearing in mind their great variation in size, probably associated with the manner in which they are matured, I am convinced that this isolate represents the species described by Phillips and Plowright and transferred by Saccardo to *Sphaeroderma* as *S. episphaeria*. The subiculum, supposed to be characteristic, may be present or absent, depending upon environmental factors, and is without taxonomic significance. The clavate asci and their early gelatinization, followed by the maturation of the spores in the gelatinous cavity of the ascocarp, and their eventual discharge in a gelatinous tendril due to the pressure of further spore formation at the base, is wholly alien to the processes of spore formation and discharge in typical members of the Hypocreales. The assignment of *Sphaeroderma* to that order would seem to be based on no more than superficial characters. The genus should eventually be grouped with other genera having the same type of ascus dehiscence.—G. W. MARTIN, State University of Iowa, Iowa City.

ENERTHENEMA BERKELEYANUM

Enerthenema berkeleyanum Rost. (= *E. syncarpon* Sturgis; *E. papillatum* var. *syncarpon* G. Lister) is a rarely collected slime-mold known

from very few localities. In the southeast, for instance, the species is recorded only from South Carolina (type).

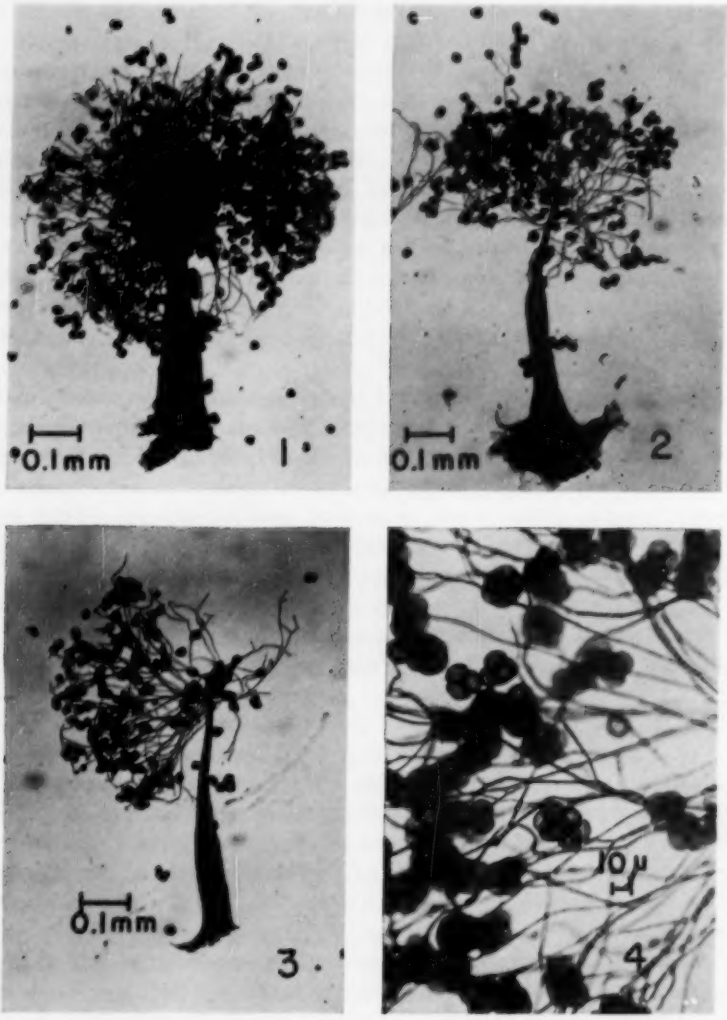
In October, 1954, a single development of *Enerthenema berkeleyanum* was found on rotting wood near Lake Brandt, Guilford County, North Carolina. The characteristics of this collection differ somewhat from the description of the species by Martin (N.A. Fl. **1**(1): 73. 1949). These differences and the fact that *E. berkeleyanum* has not, apparently, been illustrated in published literature, seem sufficient justification for a brief note on the North Carolina collection (No. DMH *1535*). The present collection was compared with exsiccati from the New York Botanical Garden (N.Y.B.G. *12622, 12623, 1019;* as *E. syncarpon* Sturgis), and from the Farlow Herbarium (collections by R. Hagelstein and E. C. Smith).

The sporangia of the North Carolina collection are rather small, jet-black (with spores), and consistently globose. The stipes are short, gradually or abruptly tapering (FIG. 2, 3), and shining-black. Although the presence of a flat or salver-shaped apical columellar disk is the distinctive generic characteristic, this disk is often lacking. Very few sporangia were observed (including those in the N.Y.B.G. exsiccati) in which the columellar disk was present. If present at all, the disk is frequently observable only by microscopic examination, when it appears as a very small, often imperfectly formed expansion at the tip of the columella (FIG. 3). In the Hagelstein collection the disk is present on all but a few sporangia.

In those sporangia which lack even a rudimentary columellar disk the capillitium arises either directly from the acuminate tip of the columella, or springs laterally from all parts of the columella. In a completely blown sporangium with a laterally-originating capillitium there is a pronounced suggestion of an imperfectly formed *Comatricha*. An "annulus" consisting of the persistent basal portion of the peridium is lacking, at least in the specimens examined. Such a peridial ring is common in *Enerthenema papillatum*.

The capillitial threads are predominantly smooth or merely slightly roughened (FIG. 4). *Enerthenema papillatum*, on the other hand, has distinctly roughened threads. In general aspect the individual threads of *E. berkeleyanum* are much more flexuous than in *E. papillatum*, and appear in blown specimens to be considerably more rigid. Hence, the capillitium of *E. berkeleyanum* does not generally exhibit the pronounced depending habit characteristic of *E. papillatum*. The clusters of 3–12 partially echinulate spores (FIG. 4) are diagnostic for *E. berkeleyanum*.

It is of passing interest to note that those exsiccati identified as



FIGS. 1–4. *Enerthenema berkeleyanum* Rost. 1–3. Sporangia. 4. Capillitium and spores.

Enerthenema syncarpon contained specimens with at most rudimentary columellar disks. Those collections identified as *E. berkeleyanum*, on the other hand, contained fructifications with well-developed and obvious disks. Other features of similarity, however, justify the presently ac-

cepted synonymy.—T. W. JOHNSON, JR., Department of Botany, Duke University, Durham, North Carolina.

LACTO-FUCHSIN: A NEW MEDIUM FOR MOUNTING FUNGI

The lactophenol mounting fluid developed by Amann in 1896 is still the choice of most mycologists for microscopic examination of molds. The long popularity of this medium attests its many desirable qualities. It has however, three serious drawbacks. First, its refractive index is close to that of the cell walls of many hyaline fungi, rendering the walls difficult to see. Second, the Cotton Blue dye customarily incorporated stains slowly and often weakly and its color is unsuitable when blue or green filters are used to increase resolution or contrast. Third, it does not solidify; thus mounts must be ringed or stored flat, and dust or immersion oil is difficult to remove without disturbing the preparation.

The first two difficulties, refractive index and weak staining, can be overcome by using lacto-fuchsin mounting medium. It is prepared by dissolving 0.1 gm of Acid Fuchsin in 100 ml of lactic acid. The acid should be as free from water as possible. Mounts made with lacto-fuchsin are similar to those made with lactophenol-Cotton Blue, but they are superior in that cell walls stand out more clearly, staining is more rapid, and the color is more suitable, especially for photography.

The third difficulty, fluidity, can be overcome by mixing lacto-fuchsin half and half with Gurr's "Water Mounting Medium" (G. T. Gurr, London, S.W. 6, England). Mounts made with this mixture become firm enough to clean after a day or two. The mixture keeps well in a tightly stoppered dropping bottle, although after some months needle-shaped crystals may appear. These can be removed by filtration through paper with the aid of suction.—J. W. CARMICHAEL, Provincial Laboratory of Public Health, University of Alberta, Edmonton, Alberta, Canada.

AGEOTROPIC SPORANGIOPHORE CONTROL FOR CULTURES OF PHYCOMYCES

For stocks of *Phycomyces blakesleanus* and of *P. theobromatus* standard practice in tube culture is very disadvantageous. The extremely long sporangiophores bring the sporangia into contact with the cotton plugs, even in five days, so that thereafter whenever the plugs are removed

sporangiospores escape in great numbers to contaminate everything nearby. Efforts to restrain sporangiophore elongation by restricting the sugar content of the culture medium have been so entirely ineffective that recourse was had to other expedients, only one of which has so far been effective.

A simple way to circumvent a seemingly inherent character in both species, which has proved entirely satisfactory in the care of three stock cultures of *P. blakesleanus* and one of *P. theobromatus*, is merely to invert the culture tubes, i.e. with the tube mouths downward. Although the sporangiophores at first grow perpendicularly away from the surface of the agar slant they soon turn backward before the sporangia are borne so that at maturity the sporangia are at the surface of the agar with the somewhat coiled and twisted sporangiophores adjacent.—SHUH-WEI HWANG, American Type Culture Collection, Washington, D. C.

THE INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

The International Society for Human and Animal Mycology was founded in Paris on July 6, 1954 by a group of scientists representing 10 different nations assembled on the occasion of the VIII International Congress of Botany. The following officers have been selected: President: P. Redaelli (Milan); Vice-Presidents: C. W. Emmons (Bethesda), G. T. Ainsworth (Exeter), P. Negroni (Buenos Aires), G. Ségretain (Paris); General Secretary: R. Vanbreuseghem (Anvers).

The objects of the Society are: to bring together qualified persons interested in the study of fungi on humans and animals; to encourage the formation of regional groups of these persons; to organize meetings of the members of the Society on the occasion of International Congresses; to publish, as soon as possible, a bulletin, devoted to human and animal mycology.

All those who wish to become members of the International Society for Human and Animal Mycology are invited to send their applications to the General Secretary, giving details of their qualifications together with a list of their scientific publications. The annual subscription has been fixed at $3.00, to be remitted to Account No. 133,700 of the General Secretary, Dr. R. Vanbreuseghem, at the Banque d'Anvers, Antwerp, Belgium.

LABORATORY REFRESHER TRAINING COURSES

The Communicable Disease Center of the Public Health Service offers again its series of refresher courses. Of particular interest to mycologists are the two courses on Laboratory Methods in Medical Mycology. Part 1, on cutaneous pathogenic fungi, is offered from October 31 to November 11. Part 2, on subcutaneous and systemic fungi, is offered from November 14 to 25. Completion of Part 1 or equivalent education or experience is a prerequisite for Part 2.

Information and application forms should be requested from Laboratory Training Services, Communicable Disease Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia.

REVIEWS

LES CHAMPIGNONS. TOME II. SYSTEMATIQUE, by Fernand Moreau (Encyclopedie Mycologique XXIII), pp. 941–2120, figs. 465–1300. Paul Lechevalier, Paris VI°. 1954. Price 11,000 fcs. (about $31.00).

The first volume of this comprehensive work was reviewed in MYCOLOGIA **45**: 536–537. 1954. The second, and even larger, volume reached the United States at about that time. It is entirely devoted to the third and final part, dealing with the systematic treatment of the fungi, and also includes the indexes to both volumes, one to authors cited and one to genera and subgenera.

After a short introductory chapter on general principles of classification and one one fungus-like organisms not regarded by the author as closely related to the fungi—Actinomycetes, colorless alga-like forms and Myxomycetes, including the Plasmodiophorales—the bulk of the volume, broken into 21 chapters and occupying 1073 pages, is devoted to a systematic treatment of the fungi by groups. A final chapter discusses the phylogeny of the fungi.

The classification adopted will seem unfamiliar to most readers. The basic division is between the MASTIGOMYCETES, including the chytrids, the water molds and the downy mildews, and the AMASTIGOMYCETES, embracing all other fungi. The Amastigomycetes are subdivided into the Zygomycetes, with the usual circumscription, the Periascomycetes (Protascales, Protomycetales, Ashbyales, Spermophthorales, Dipodascales, Synascales) and the Dangeardiomycetes. The last-named group, characterized by the usually septate mycelium, with its compartments (the author specifically recognizes that these are not homologous with "cells" as usually defined) often uninucleate, the development of distinct fructifications and the occurrence, often accompanied by accessory spore forms, of spores produced in asci or on protobasidia or basidia. This enormous group, comprising the great bulk of the fungi, is further subdivided as follows:

Simple Ascomycetes: Endomycetales, Saccharomycetales, Taphrinales
Carpoascomycetes
  Aspergillales
  Ascoloculares: Uleomycetales, Pseudosphaeriales, Hemisphaeriales, Erysiphales

Lagynocarpous Ascohymeniales: Sphaeriales, Diaporthales, Valsales, Coronophorales, Clavicipitales, Laboulbeniales
Discocarpous Ascohymeniales: Pezizales, Ostropales, Helotiales, Lecanorales, Tuberales
Protobasidiomycetes: Uredinales, Ustilaginales
Dangeardiomycetes intermediate between Protobasidiomycetes and Eu-Basidiomycetes: Auriculariales, Tremellales, Dacryomycetales, Tulasnellales
Eu-Basidiomycetes: Aphyllophorales, Boletales, Agaricales, Gasteromycetales

The Fungi Imperfecti are regarded as a temporary group based on lack of knowledge but are given adequate attention, major classification being based on spore formation or lack of it. The pycnidial and acervulate forms are thus reduced to families of the Conidiospori.

The chapter on phylogeny is well worth careful study. Admitting that the inadequacy of the fossil record makes any such discussion hypothetical, the author brings together the considerations which underlie his treatment. He regards the fungi as derived from a flagellated protozoan ancestry through four independent phyla, the Prosomastigochytridiales, with a single anterior flagellum, and no forms developing beyond the chytrid level; the Opisthomastigochytridiales, with a single posterior flagellum, leading to the Blastocladiales and the Monoblepharidales; the Dimastigochytridiales, with two flagella, leading to the Saprolegniales, Peronosporales, etc.; and the Amastigochytridiales, the non-flagellate forms, which are presumed to be ancestral to the Zygomycetes, the Periascomycetes and the Dangeardiomycetes.

In the present state of the classification of the fungi, any author is justified in presenting what he regards as an improvement on the current systems. When such a scheme is as fully documented and as carefully thought out as is Moreau's, it is entitled to the fullest and most careful consideration. While it seems unlikely that mycologists will accept the concept of the Dangeardiomyces for all the Basidiomycetes and the great bulk of the Ascomycetes without extremely careful consideration of the known facts, the suggestion itself should lead to reexamination of many widely held but inadequately based assumptions.

The literature cited is impressive in its bulk alone; it is perhaps inevitable that some papers have been overlooked which might have modified certain of the author's conclusions. It is also inevitable that a few errors of fact should have crept in—none, so far as noted, of fundamental importance. Since this promise to be an important reference

work for years to come, such errors will undoubtedly receive due notice as specialists attack the problems involved.

These volumes should be available to students in every laboratory where fungi are studied seriously for their own sake rather than for their immediate applied importance.—G. W. M.

ILLUSTRATED GENERA OF IMPERFECT FUNGI, by H. L. Barnett. 218 pp., 302 figs. Multilithed. Burgess Publishing Co., Minneapolis, Minn., 1955. Price $4.00.

This is not intended as a monograph of the imperfect fungi, but as an aid to teaching and as a ready means for identifying to genus a substantial number of the imperfect fungi encountered on living plants, dead plant parts, in soils, and many other habitats where fungi are found. Of the 302 genera described and illustrated, 283 belong in the Imperfecti and 19 are Phycomycetes whose habit of growth is such that they might readily be taken for imperfect fungi. The number of drawings is greater than the number of figures suggests, since most of the figures are composed of several different drawings, showing appearance at different magnifications, sections where that will be helpful, relations to host in the case of parasitic forms, variation and similar features. In connection with many of the generic descriptions, reference is given to a recent treatment where further information may be sought. A key is given to all genera described and illustrated.

Even in modern comprehensive works on the fungi, it has been customary to regard the Fungi Imperfecti as a strictly temporary group to be discarded as soon as the perfect stages are known, usually with the implication that such knowledge is imminent. It is now recognized that even when the perfect stages are known, the imperfect stages must be treated, for very practical reasons, as biological entities. It is beginning to be suspected that many of them which may once have had a perfect stage have long since discarded it, and a few heretics are permitting themselves to wonder whether some ever had it. In any event, the renewed interest in these forms is demanding that they be given greater attention than has been customary in courses devoted to the fungi. Barnett's book promises to be extremely useful, not only in classes, but in the very many laboratories where imperfect fungi are of concern.—G. W. M.

STUDIES ON THE ECOLOGY OF YEASTS, by Aage Lund. 132 pp., 30 figs., paper. Einar Munksgaard, Copenhagen. Price 20 Dan. kr. (about $2.90).

This is essentially an ecological study of the occurrence of yeasts, both sporogenous and asporogenous, in the Copenhagen area. Isolations were made from flowers, fruits, root crops, agarics, tree exudates, insects, leaves and bark, dung, and soil. Of the 56 species isolated, over 43 were members of the Crytococcaceae. A number could not be determined to species and may be undescribed. As might be expected, sweet fruits and fleshy fungi yielded large counts. Only about 25% of the flowers investigated proved to have large numbers. Soil contained relatively small numbers and these may have been largely due to infiltration from the surface of cells developing on more favorable substrata. It is suggested, however, that the soil is important as a winter reservoir. Growth of bacteria and molds was checked by the addition of 0.25% sodium propionate to hopped wort agar. This medium had little effect on yeast colonies. Findings on seasonal distribution and effect of external factors are included.—G. W. M.

MANUSCRIPT

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